"Coliform Source Tracking Methods (Multiple Antibiotic Resistance and Coliphage Typing) and Presumptive TMDL Modeling to Identify Pollution Source in Selected SC Watersheds"

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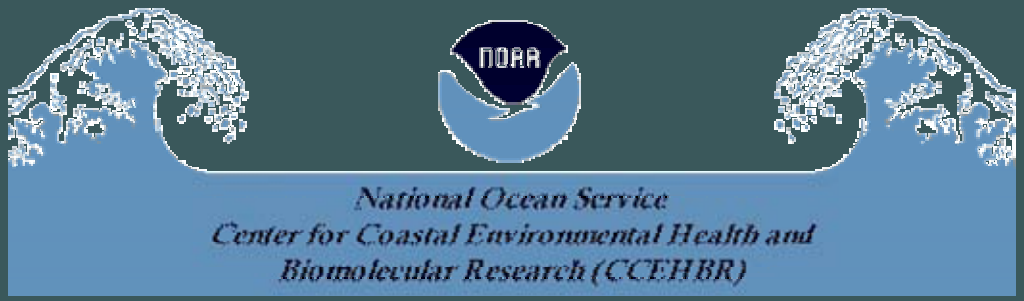

Collaborators

- ***U.S. Dept. of Agriculture, Eastern Regional Research Center***

  Dr. M. L. Tamplin

- ***NOAA, NOS, Coastal Center for Environmental Health and Biomolecular Research***

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  H. F. Cantrell

Fecal Coliform Bacterial Sources: Estuaries

- **Human Sources**
  - Septic systems
  - Wastewater treatment plants
  - Marinas
  - Combined sewer overflows
  - Golf Courses
- **Non-human Sources**
  - Urban: domestic animals & urban wildlife
  - Rural: livestock & rural wildlife

Why Should We Be concerned:

Microbial Contamination

- **Recreational Use**
  - **Increase in infection from contact recreation**
- **Tourism**
- **Shellfish Harvesting**
  - **Contaminated shellfish meat**
  - **Shoreline Survey and Monitoring (ISSC)**
  - ***Need to Discern Pollution Sources***

Pathogens:

Norwalk virus, Hepatitis, Cryptosporidium, Vibrios,

E. coli

Possible Human Sources

Illegal dumping
from boats

Sewage: sewer system,
septic tanks

Possible Animal Sources

Wildlife

Agricultural animals

Pets

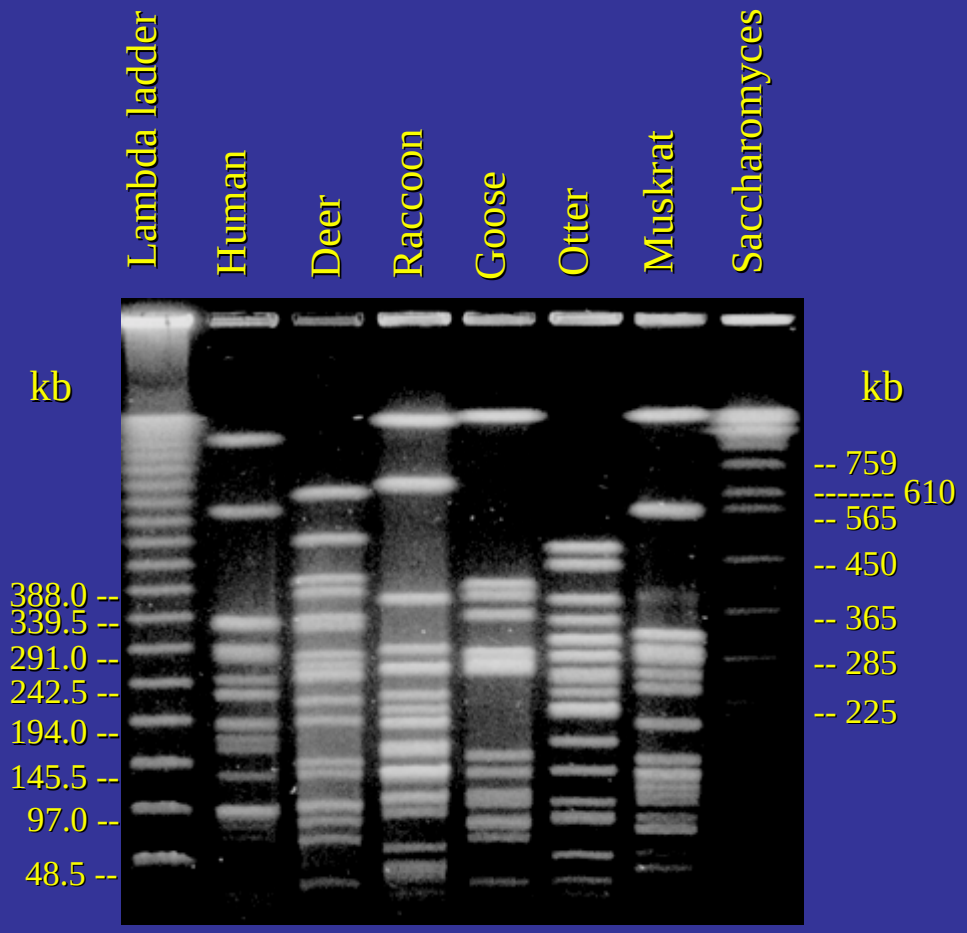
SUMMARY & OVERVIEW: *MST Methods*

- **Bacterial Indicators**
  (*E. coli*, Enteroccocus, and *Bacteroides*)
  - *Phenotypic* - ARA, Carbon Source Profiles, Etc.
  - *Genotypic* - RT, PFGE, Rep/BOX PCR, LH-PCR, T-RFLP, AFLP, Etc.
- Biomarkers - Enterotoxins, sIgA, Etc.
- **Viral Indicators & Pathogens**
  (Coliphages, Enteroviruses, Adenoviruses, and HAV)
  - Phentoypic/Genotypic - Coliphage Serotyping and Genotyping
  - Genotypic - RT-PCR & Nested PCR

MST SUMMARY & OVERVIEW: *Bacterial and Viral Research Issues*

- Factors To Consider in Evaluating MST Methods
  - Accuracy
  - Sensitivity & Specificity (Human vs. Animal or Human vs. Specific Non-Human Animals
  - Ease of Methods, Training Required, & Technology Transfer
  - Cost
  - Equipment: Investment, Throughput, Automation
  - Library vs. Non Library Methods

Management of Urbanization Impacts: Coliform Source Identification



Pulsed Field Gel Electrophoresis of *E. coli* Isolates from Various Animal and Human Sources, *Not* I digests. Other methods: Ribotyping, Coliphage &Antibiotic Resistance = "Weight of Evidence" Approach

MST Issues:
Database Dependant Methods

- ***ARA, RT, PFGE***
  - Database Size
  - Accuracy of Knowns (Stool Samples vs. WWTP)
  - # of Hosts for Knowns
  - # of Isolates/Sample
  - Temporal/Spatial Issues
  - Internal vs. External Reliability & Accuracy
  - Stability (Both Isolate Level and Population-Host Level)

MST Research Issues:
Non Database Dependant Methods

- ***Biomarkers, Coliphage, Adenoviruses, Enteroviruses***
  - Accuracy & Sensitivity (Selectivity) of Knowns
  - # of Hosts for Knowns
  - # of Isolates/Samples
  - Temporal/Spatial Stability (Less Sensitive but More Universal in Geographic/Temporal Comparisons)

MST Research Needs

- **Linking MST with Waste Load Allocations**
- **Presumptive TMDLs**

  -Human*

  -Pets and Livestock*

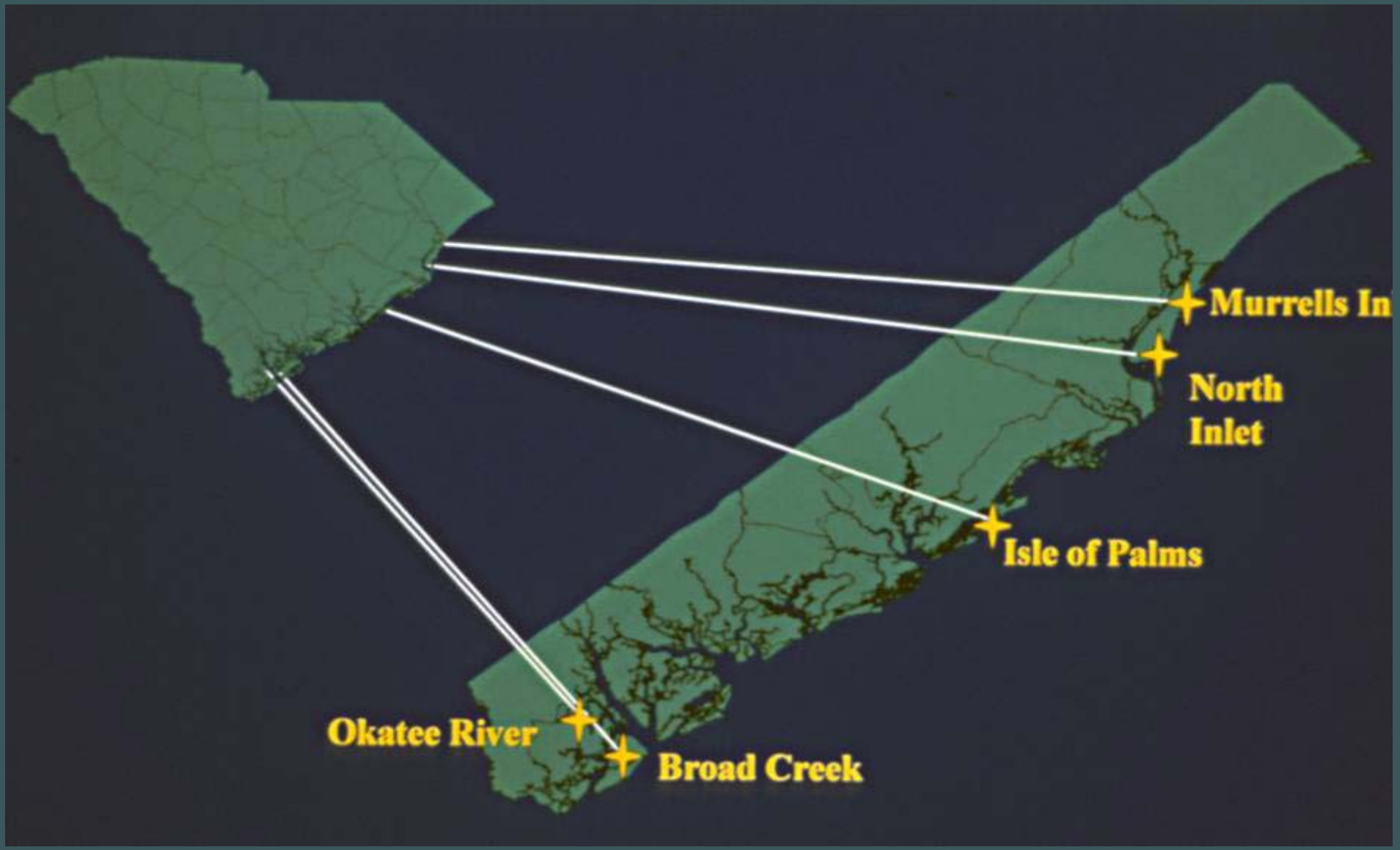
  -Wildlife
- **Case Study in SC Watersheds**

(* = Presumptively Predicted)

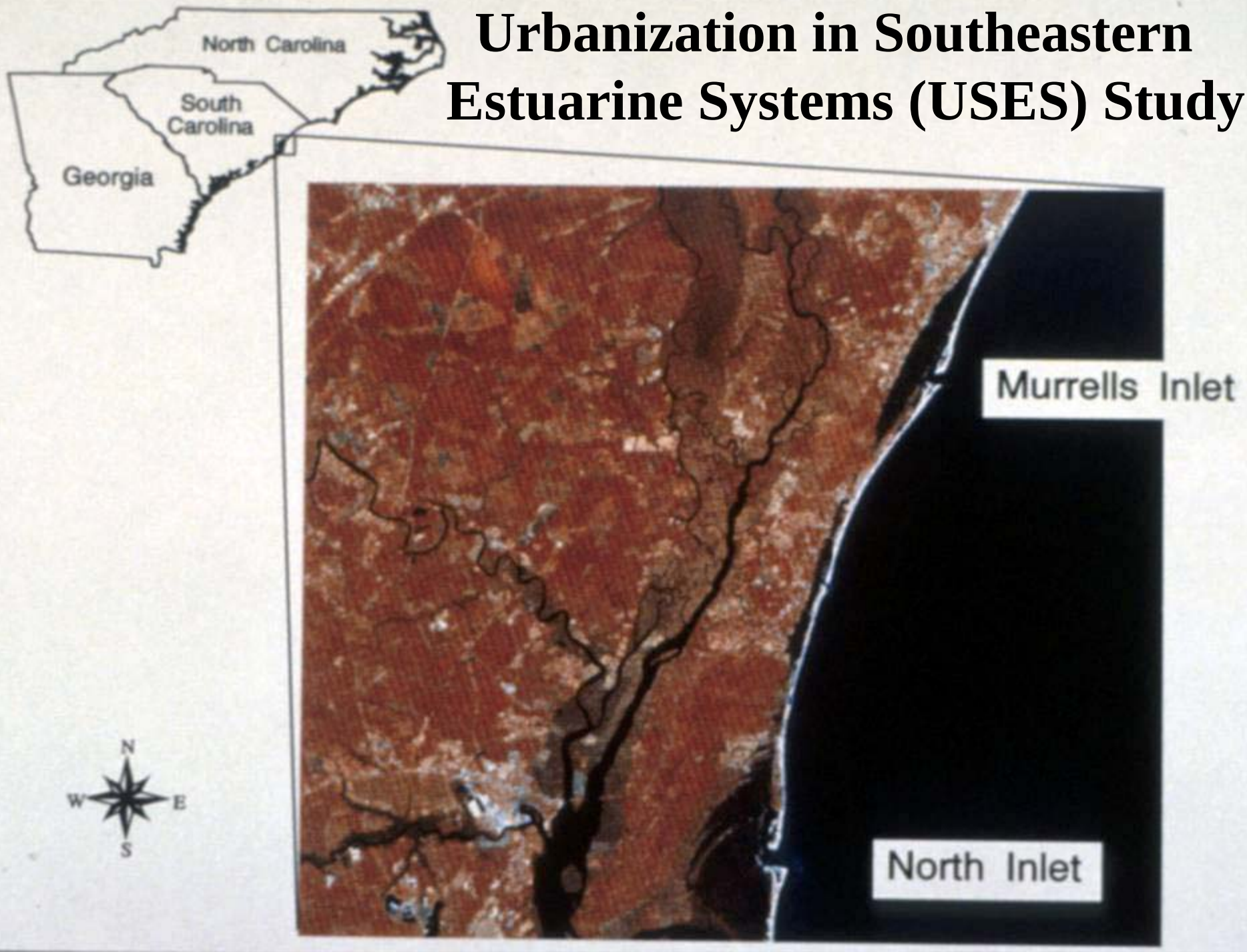
Talk Overview

- Urbanization in SE Estuaries Study (USES)
- Broad Creek - Okatee River Study
- Land Use and the Coastal Environmental Study (LUCES)
- SC Impaired Watershed Study

Site Locations Studied Within South Carolina



Urbanization in Southeastern Estuarine Systems (USES) Study



Murrells Inlet, South Carolina

1963

1984

Murrells Inlet, South Carolina

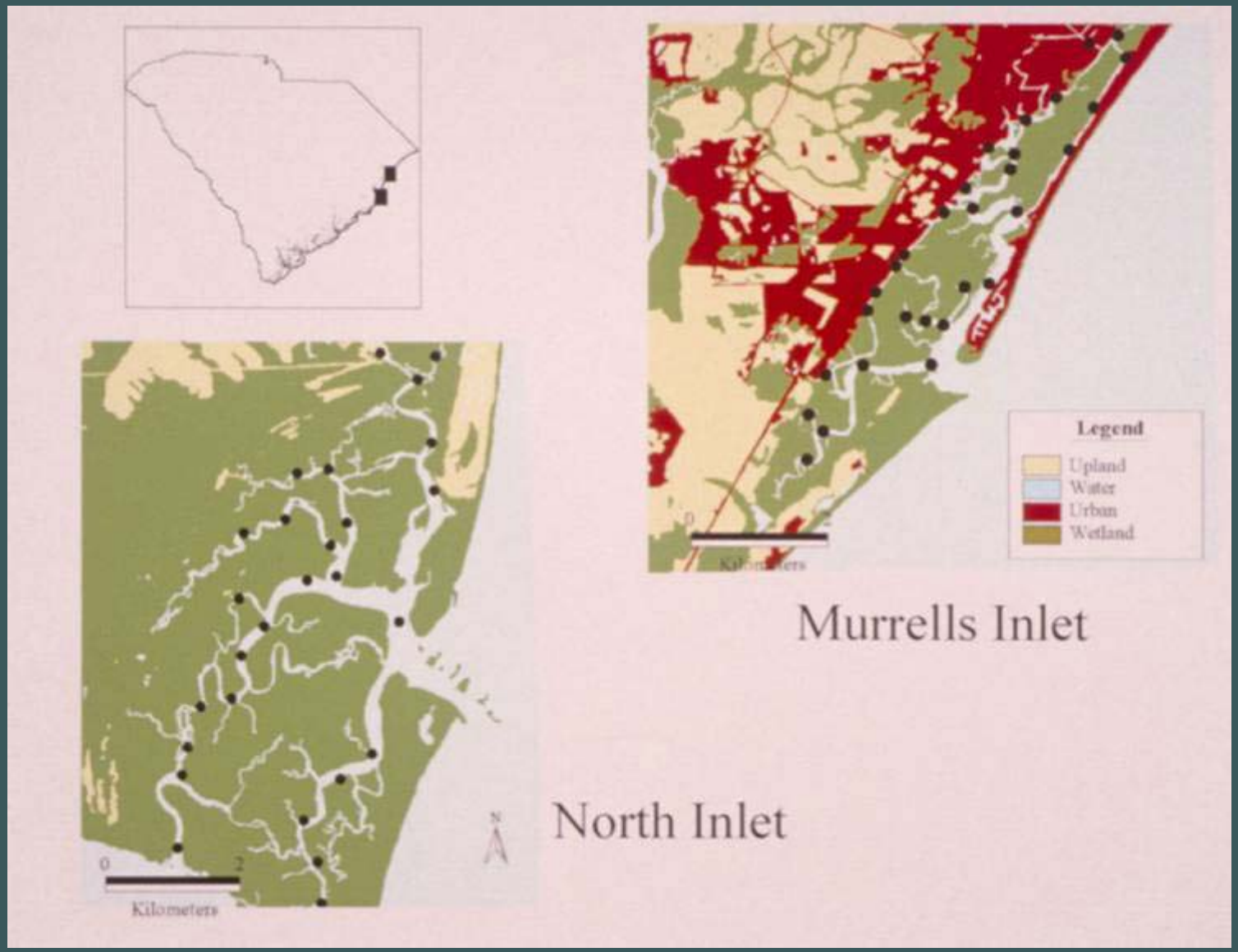
1994 **1997**

Urbanization and Southeastern Estuarine Systems Project

USES Study

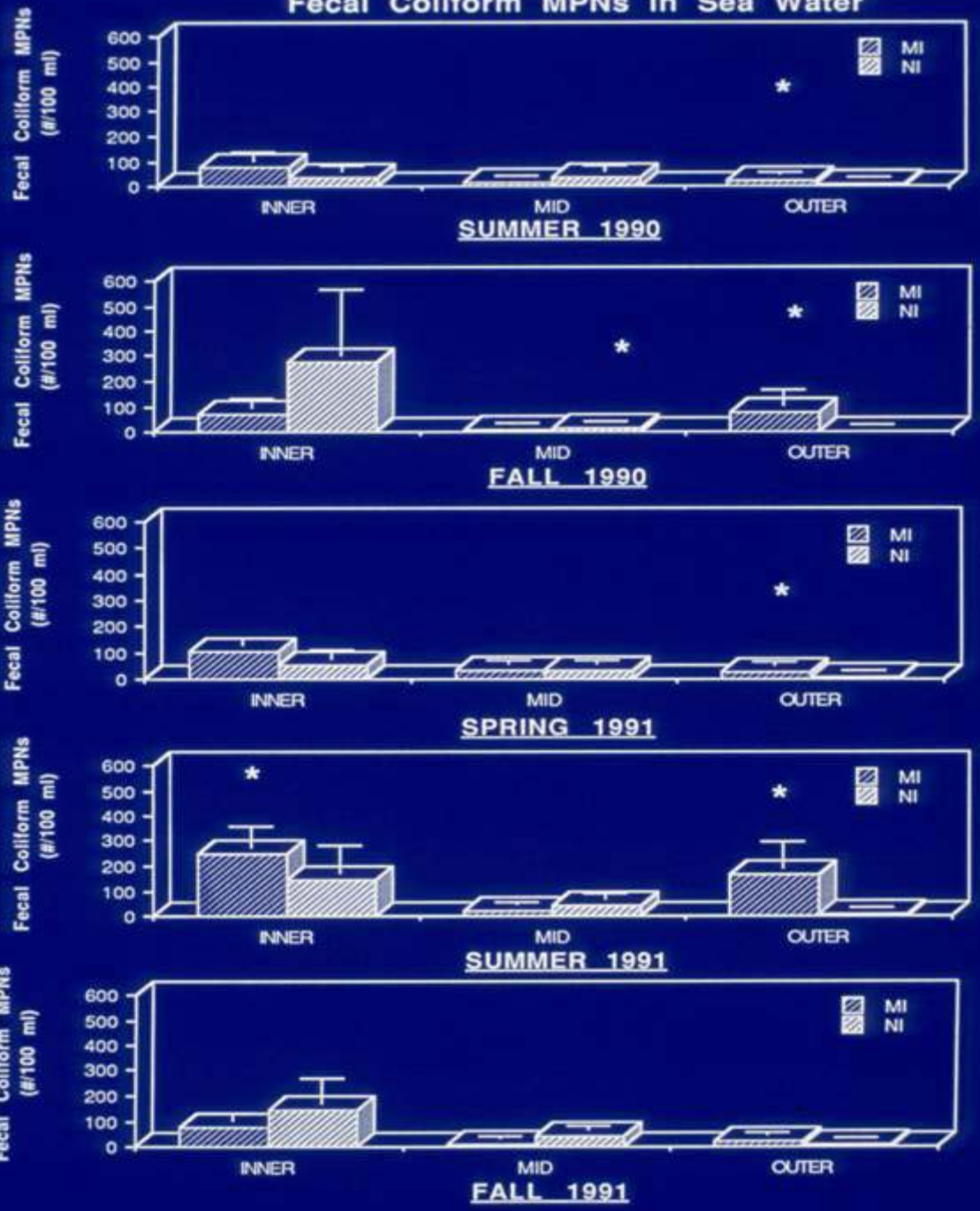
- **Objectives**
  - **Comparative Microbiology in a pristine (NI) and a highly urbanized (MI) watershed**
- **Methods**
  - **Fecal Coliform MPNs and API Typing**
  - **Surface water & oysters at 30 sites/estuary across a gradient (inner, mid and outer)**

USES Study Sites Along South Carolina Coast

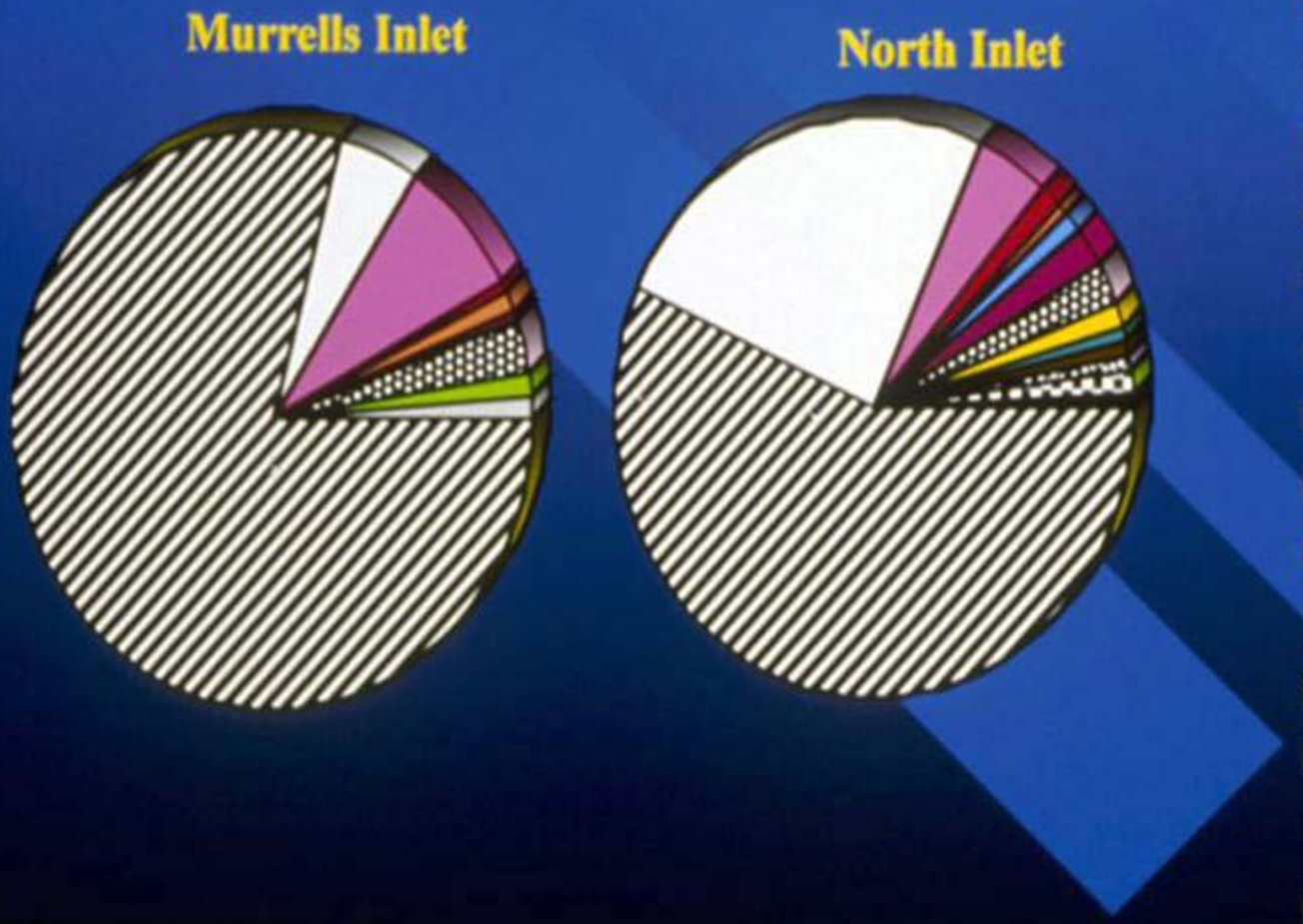


USES BACTERIOLOGY

Fecal Coliform MPNs in Sea Water



Bacterial Species Present In Water Samples At Spatial Sampling Stations (Annual Summary 1992-1993)

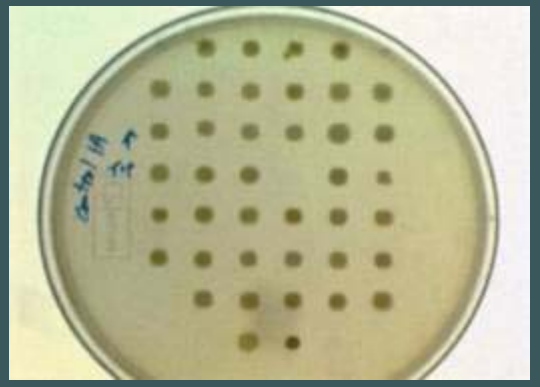


Differentiating Human & Non-human Sources of Fecal Pollution

Multiple Antibiotic Resistance (MAR) Analysis of *E. coli*

- Expose *E. coli* to different antibiotics
- *E. coli* from human sources likely to exhibit greater frequency of resistance to a greater number of antibiotics and in different patterns

Penicillin

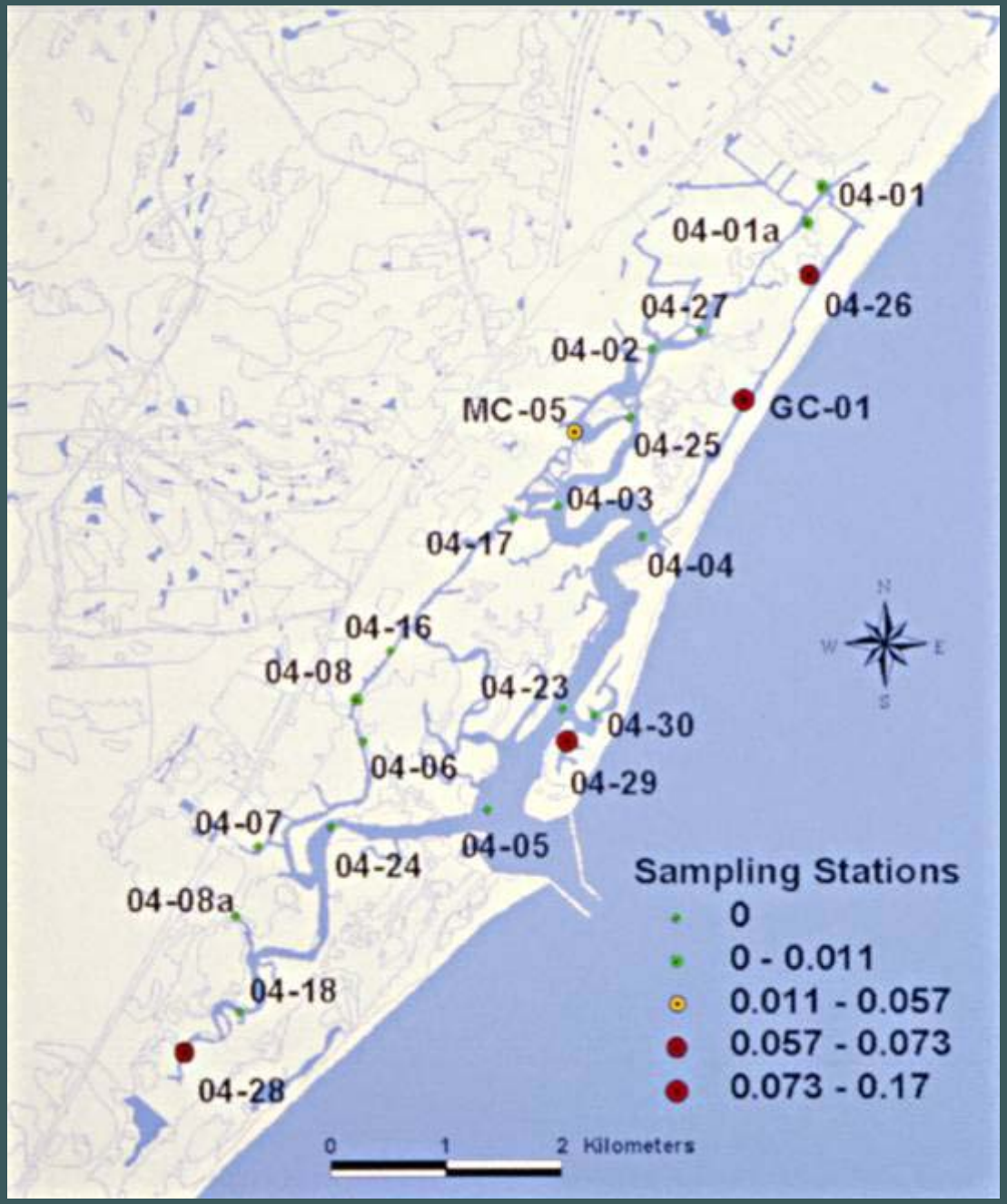


Control

Discriminant Analysis of MAR Profiles of Rookery Bay Isolates (Parveen et al., 1997)

Source (# isolates)		No. (%) of Isolates Classified As: Human	Nonhuman
Human	(111)	103 (93%)	8 (7)
Nonhuman	(104)	27 (26)	77 (74%)

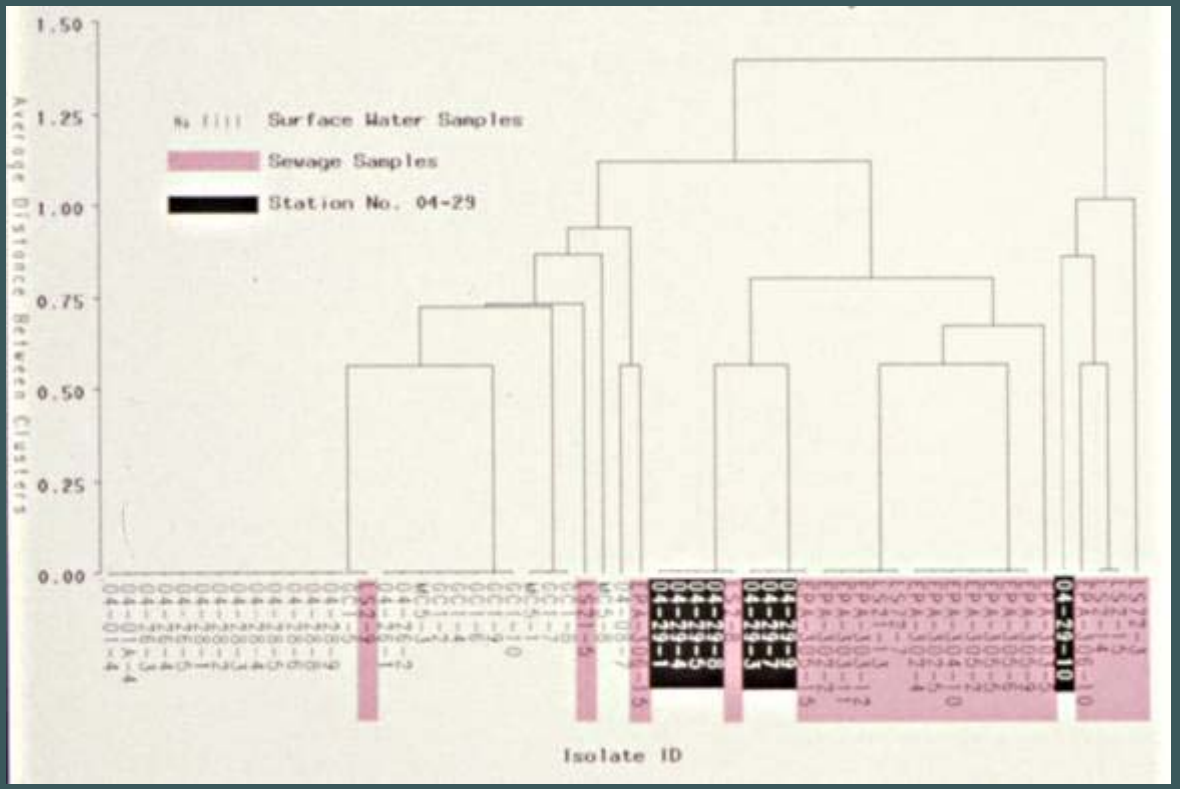

HORRY COUNTY
GEORGETOWN COUNTY
MURRELLS INLET
N
LEGEND
WATER
TIDAL MARSH
AREAS W/ SEPTIC TANKS
ROADWAYS
MAJOR HIGHWAYS



MAR Analysis of *E. coli* in MI

- Only 4 sites had high MARs (>0.05)
- Only station 04-29 had resistance to 3 or more antibiotics.
- Antibiotic resistance at all other surface water sites was to either ampicillin and/or penicillin.
- MARs of STP lift stations in MI averaged 0.07
- 78% of the STP isolates were resistant to 3 or more antibiotics compared to only 3% of the surface water samples.

MAR Resistance Pattern Cluster Analysis



Cluster analysis of antibiotic resistance patterns in surface water and STP in MI

- Only surface water station 04-29 had isolates which were highly clustered with MAR patterns in STPs lift stations.
- This was the only surface water sampling site which had distinct human MAR resistance patterns.
- All the other surface water stations (unshaded) were clustered far to the left and had MAR patterns disimilar to known human pollution sources in the area.
- Suggests that most of the pollution sources in MI were animal rather than human pollutions sources.

Total Maximum Daily Load Estimates for Fecal Coliform Bacteria: Shellfish Harvesting Waters

- Total fecal MPN Budget
  = MPN (#/100 ml) x Estuary Volume (ml)
- MPN (long term monitoring data)
- Volume (bathymetry data)
- Calculate Fecal Wasteloads (humans + domestic animals + wildlife)

Estimated Source Loadings of Coliform Bacteria in Murrells Inlet

Human Population	Estimated #		Fecal Coliforms Per Day		
	Dog	Cat	Dog	Cat	Both
19,816	3993	4472	1.33×10^{13}	2.40×10^{14}	2.53×10^{14}

Estimated Fecal Coliform Budget for Murrells Inlet

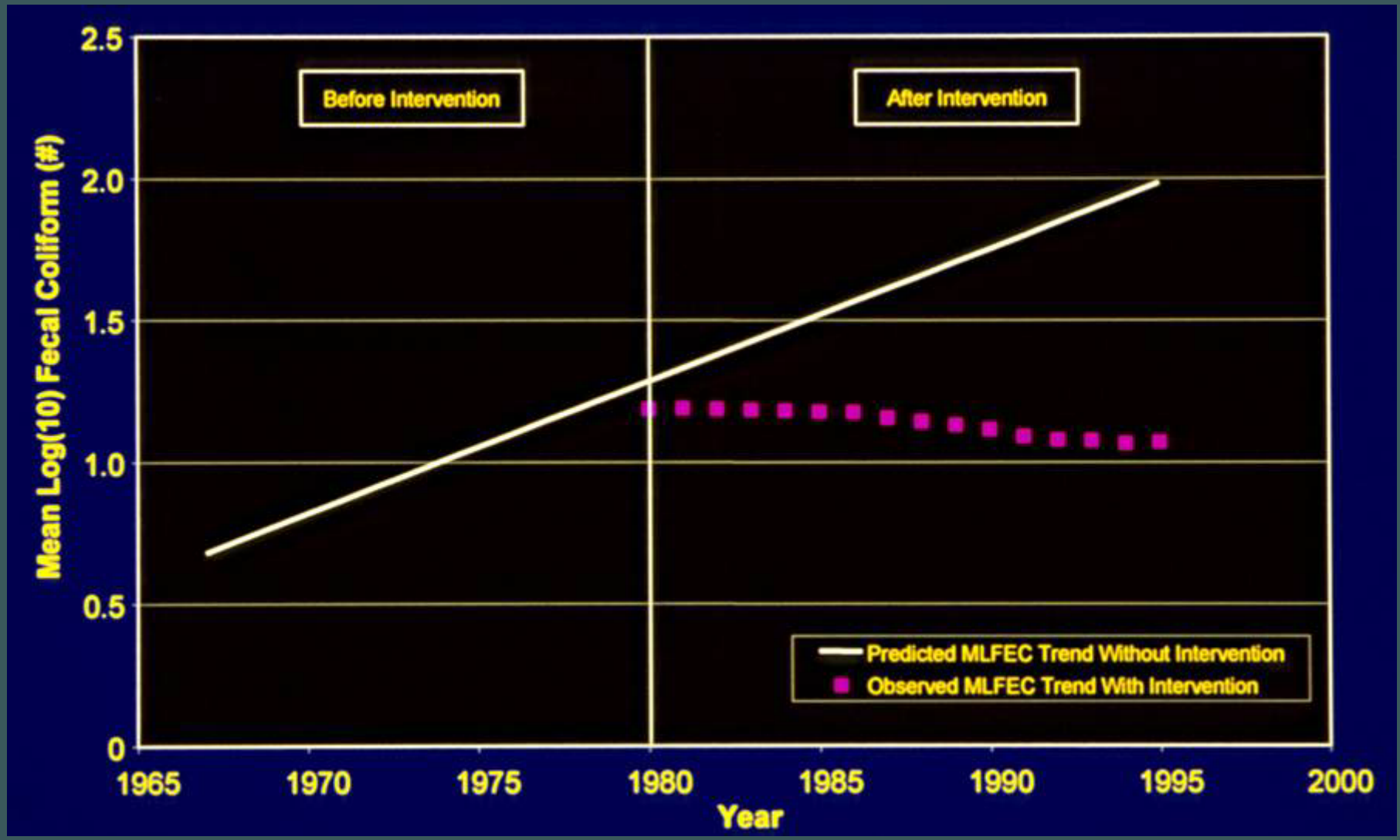
Volume Estimate [a] Coliform MPN (ml)		MPN Density [b] (#/100ml)		Estimated Fecal Coliform for MI in Total MPN
Low	High	Low	High	
1.87×10^{13}	1.06×10^{14}	12.2	133.3	2.87×10^{12} to 1.41×10^{14}

a = Volume estimate taken from high resolution GIS bathymetry survey of MI
b = MPN estimate taken from SC DHEC 10 year data set on monthly sampling, 1989-1999

Example TMDL Wasteload Calculation: Murrells Inlet

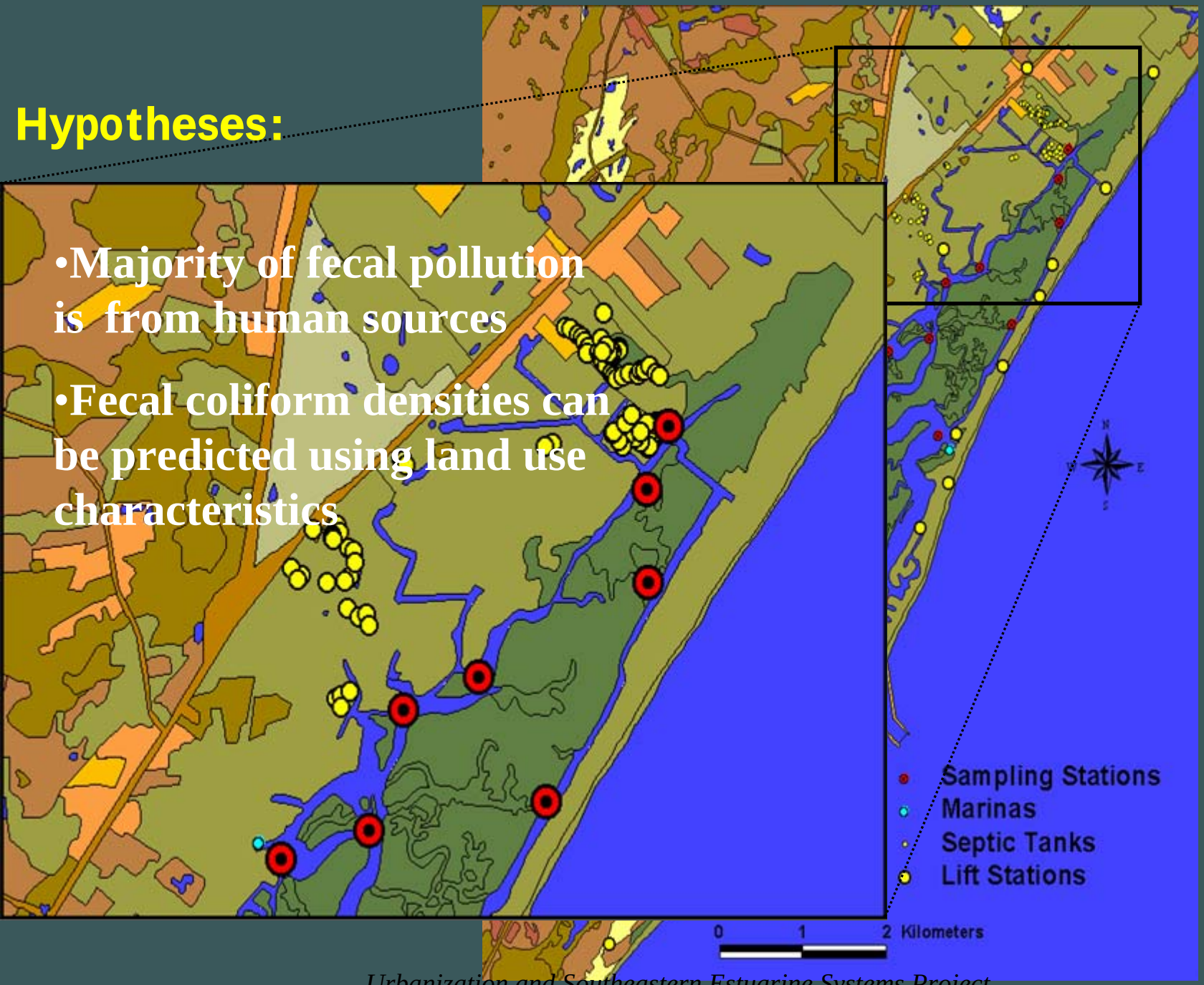
- **Human**
  - **All (19,819) = 0.43 x 10^{14} MPNs/day**
  - **Septic Tank (1,585) = 0.03 x 10^{14} MPNs/day**
- **Domestic Animals**
  - **Dogs (3,993) = 1.33 x 10^{14} MPNs/day**
  - **Cats (4,472) = 2.40 x 10^{14} MPNs/day**
- **Total Human and Pet Wasteload = 2.56 x 10^{14} to 2.99 x 10^{14}**
- **Unknown ?? = wildlife, decay rate, fecal leeching rate, groundwater inflow rate**

Intervention (by Sewage Treatment Plant) Effect on Fecal Coliform Densities at Murrells Inlet (1967-1995)



Hypotheses:

- Majority of fecal pollution is from human sources
- Fecal coliform densities can be predicted using land use characteristics



Results of regression modeling

Range of R^2 = (0.4120 - 0.4847)

Important environmental variables:

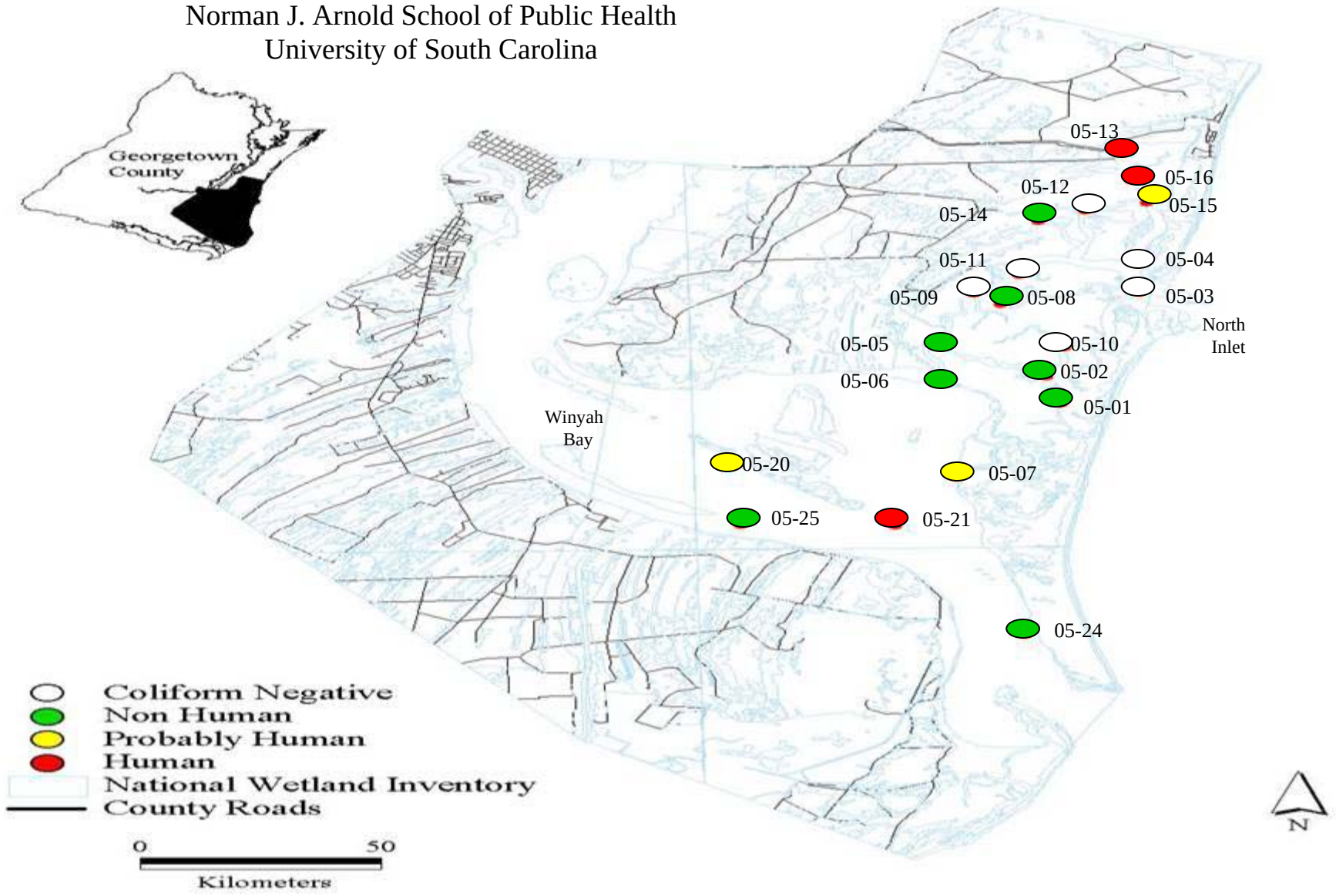
- **48-hour and 14-day rainfall,**
- **Tide, and**
- **Salinity**

Important land use variables:

- **Distance to urban areas,**
- **Distance to areas with septic tanks,**
- **Distance to lift stations, and**
- **Distance to marinas**

North Inlet MAR for May 2001

Grosso Master Thesis, Summer 2001
Norman J. Arnold School of Public Health
University of South Carolina

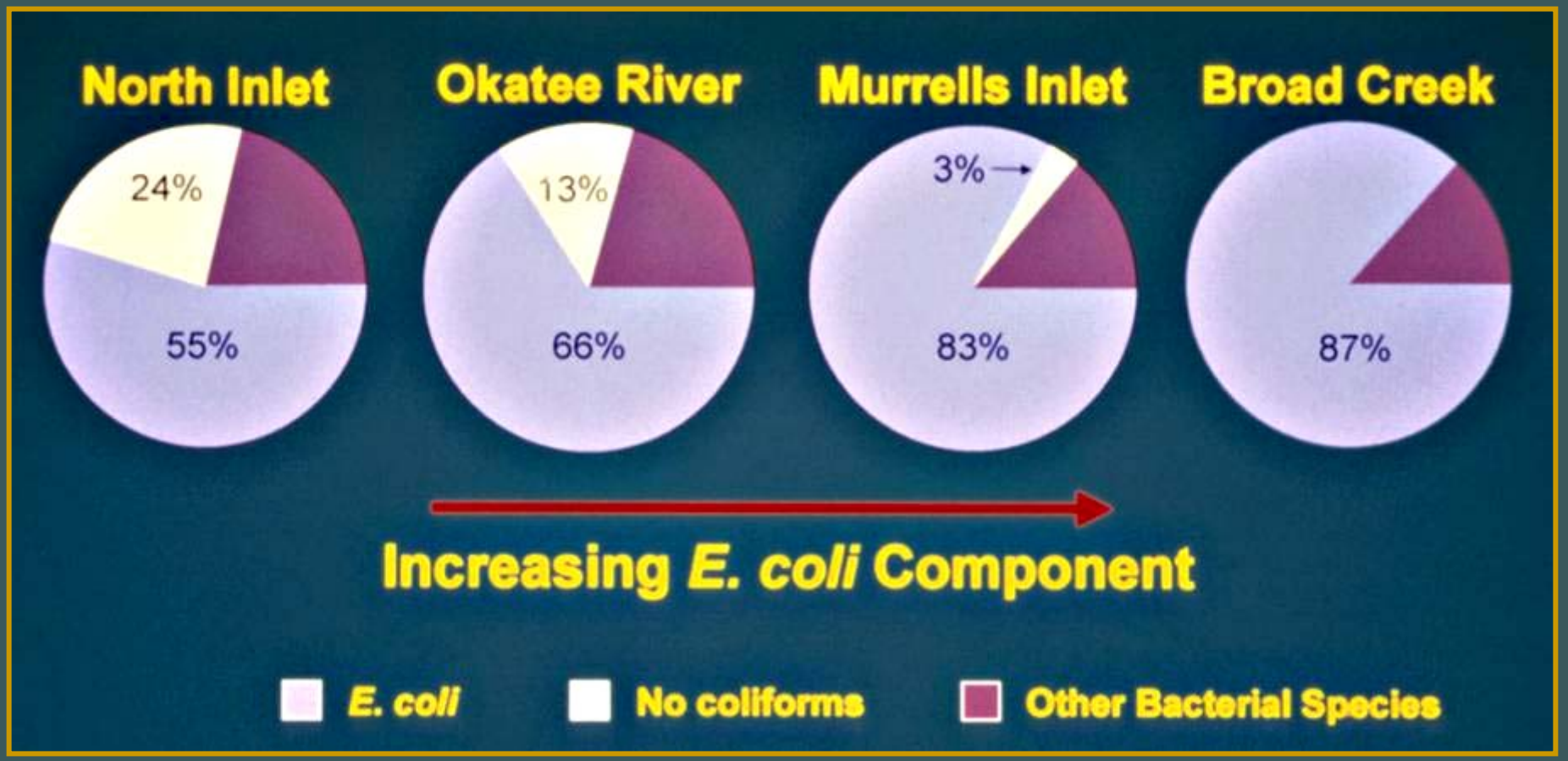


Uses Study: Conclusions

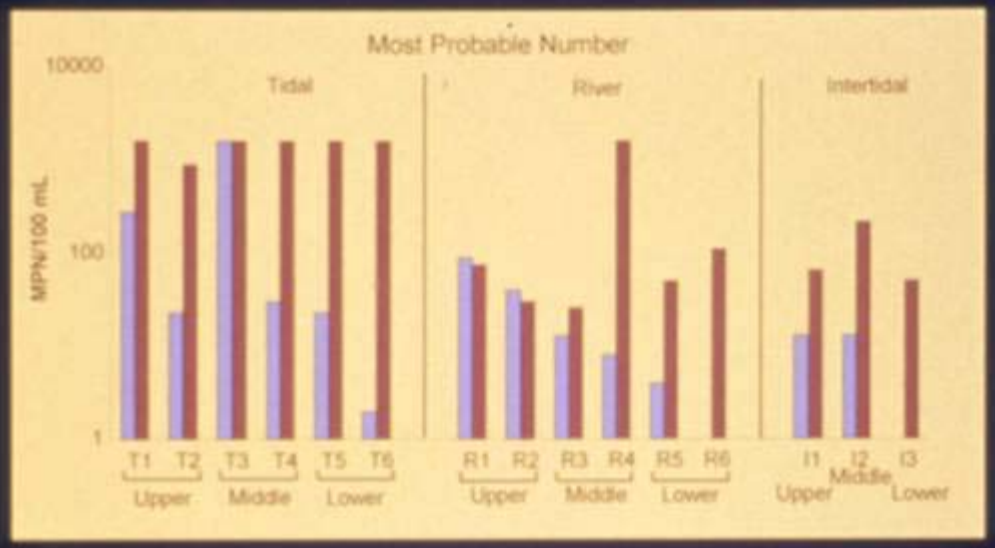
- **MARs found throughout MI and only at urban sites in NI.**
- ***MAR Analysis*: Only 1 MI site matched MAR patterns of WWTP.**
- ***GIS Analysis*: The 1 MI site with high MAR that matched WWTP was adjacent to lift station.**
- ***FC MPN Budget*: Pets > Human Input (All=17%; Septic 0.1%) = *Suggests Primarily Nonhuman Sources***

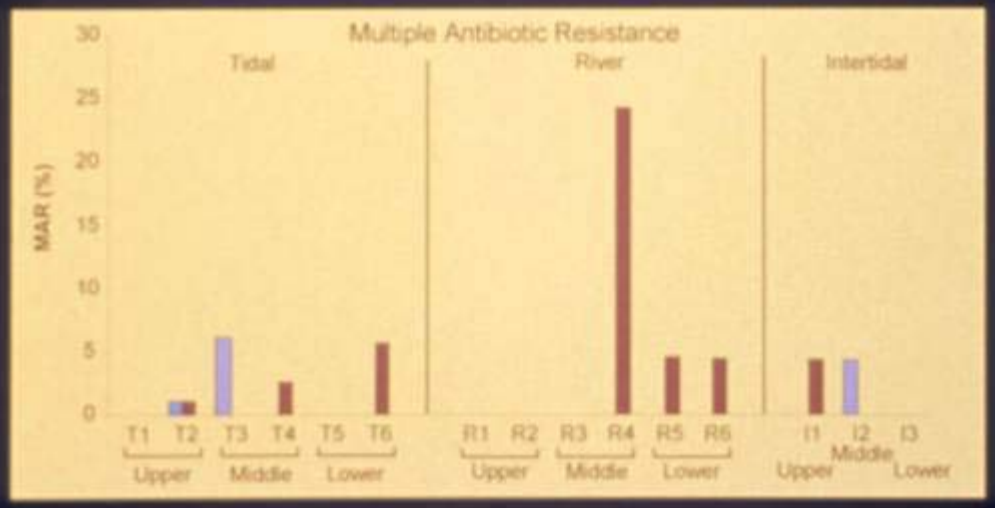
Broad Creek and Okatee River: Study Areas

Coliform Bacterial Composition in Surface Waters of Various South Carolina Estuarine Systems



Coliform Bacteria (MPN) and Multiple Antibiotic Resistance (MAR) Results





Okatee River Broad Creek

MPNs

-High MPNs (>43/100 ml) at all BC Sites

-MPNs at BC > OR

-Tidal Creeks > River or Intertidal Sites

MAR

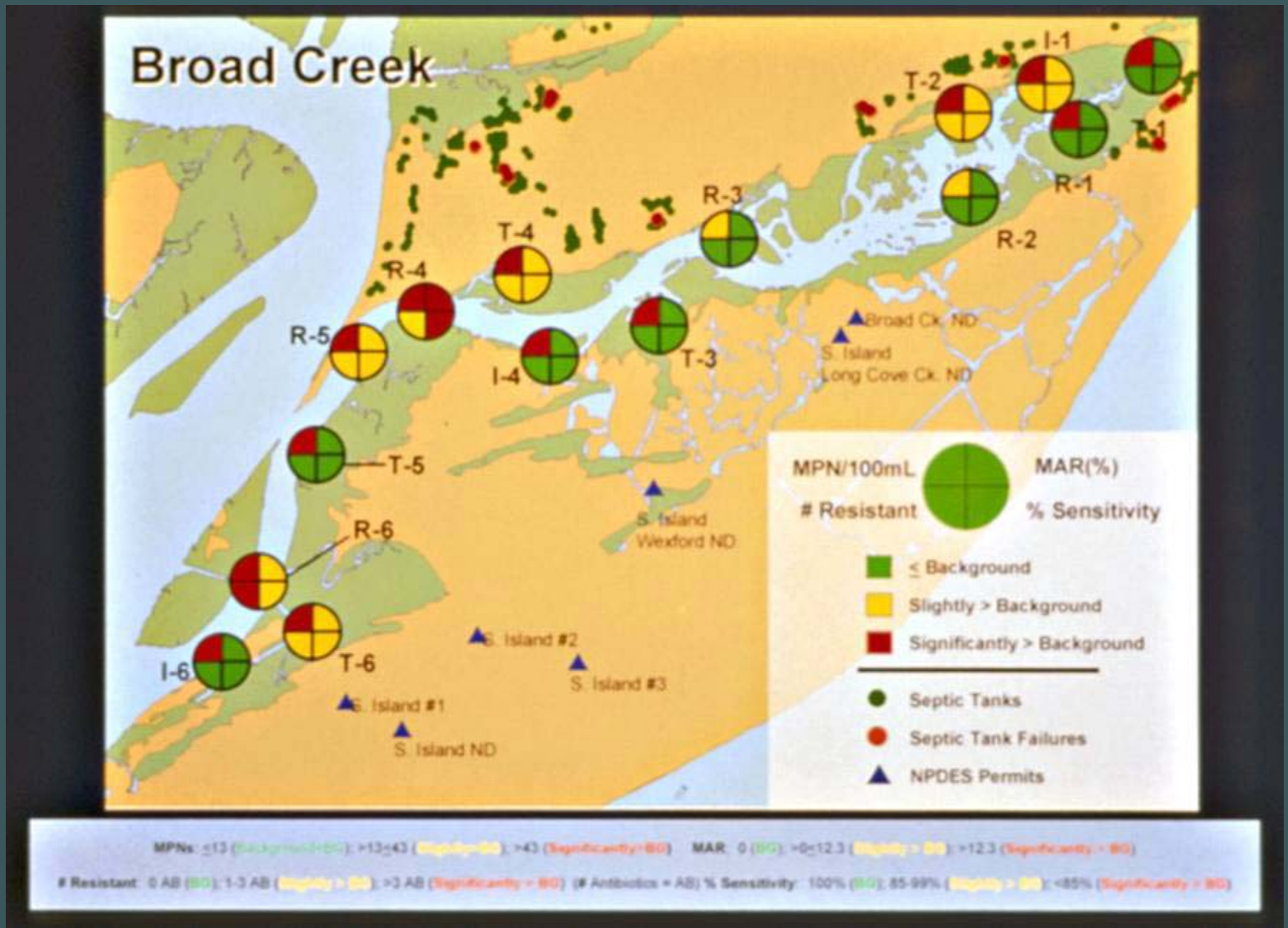
BC: 7/15 (47%) sites Positive MAR

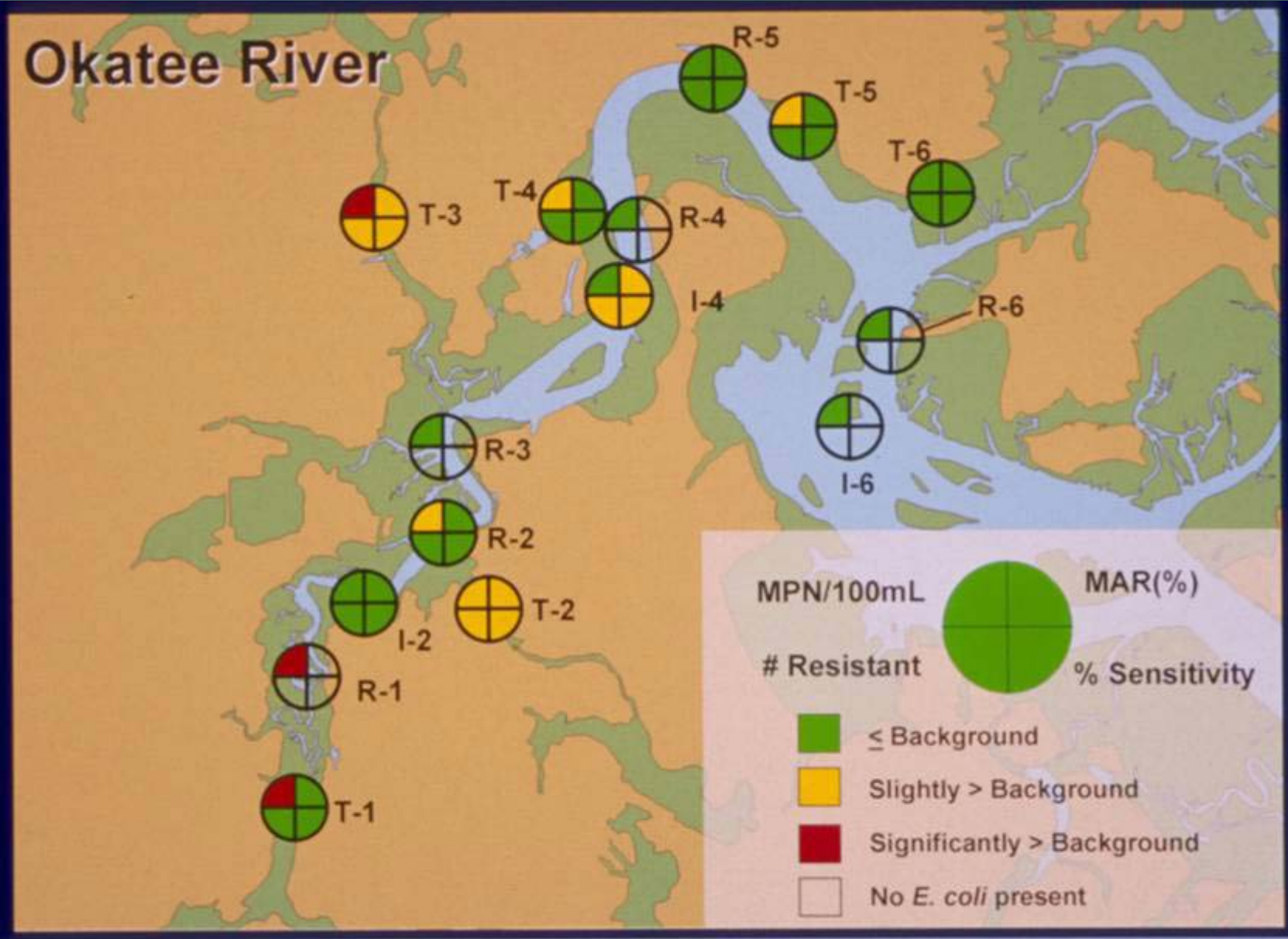
OR: 3/15 (20%) sites Positive MAR

Summary of Antibiotic Resistance in SC Sewage Treatment Plants

Antibiotic	FTSTP FIN. 12/97 (n=2)	BC-1 (n=13)	HH-1 (n=15)	LC-1 (n=15)	OK-1 (n=15)	SI-1 (n=15)	WX-1 (n=13)
Ampicillin	0	3	3	2	9	1	0
Chlortetracycline	1	0	0	0	0	0	0
Kanamycin	0	0	0	0	0	0	0
Nalidixic acid	0	0	0	1	0	0	0
Neomycin	0	4	4	1	0	0	0
Oxytetracycline	1	10	7	8	9	3	9
Penicillin G	0	3	4	1	0	0	0
Streptomycin	1	2	3	1	0	0	0
Sulfatazole	0	3	3	1	2	3	0
Tetracycline	1	3	2	1	0	0	0
Total # Resistance	4	28	26	16	20	7	9
Percent Resistant	20%	22%	17%	11%	13%	5%	7%
# Antibiotics Resistance	4	7	7	8	3	3	1

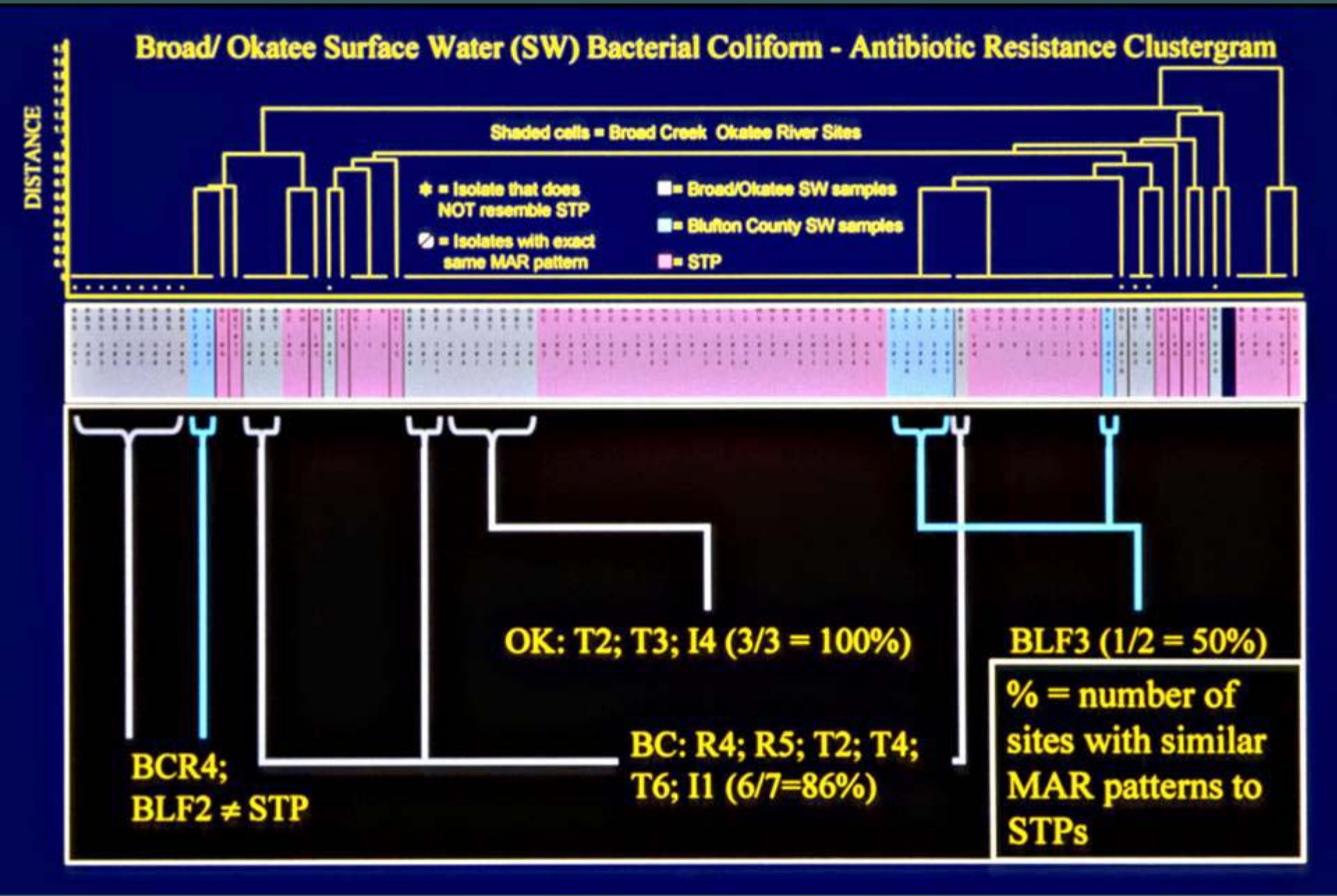
Broad Creek: MAR Results





MPNs: ≤13 (Background=BG); >13≤43 (Slightly>BG); >43 (Significantly>BG) **MAR**: 0 (BG); >0≤12.3 (Slightly > BG); >12.3 (Significantly > BG)

 # Resistant: 0 AB (BG); 1-3 AB (Slightly > BG); >3 AB (Significantly > BG) (# Antibiotics = AB) **% Sensitivity**: 100% (BG); 85-99% (Slightly > BG); <85% (Significantly > BG)

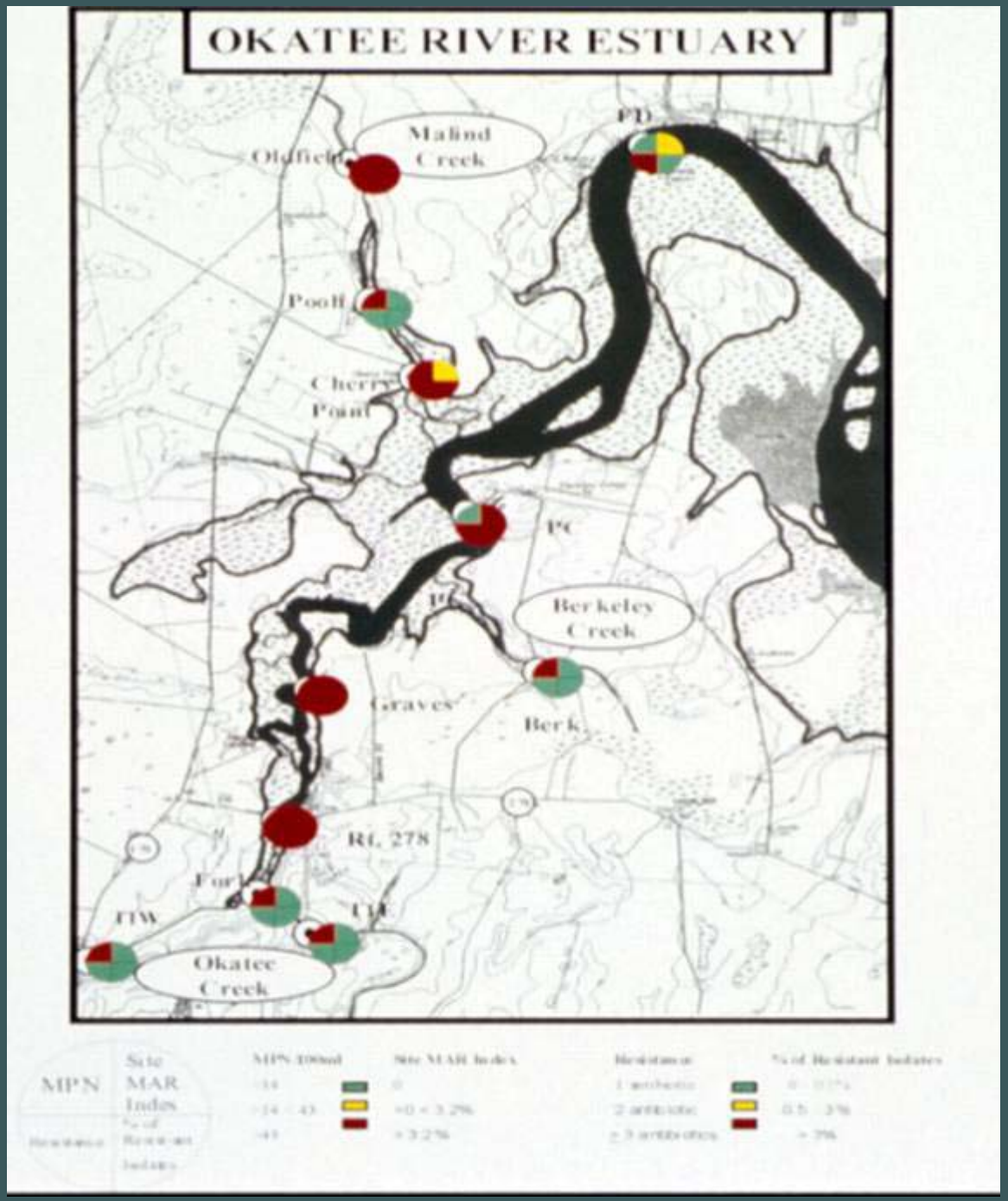

Broad/ Okatee Surface Water (SW) Bacterial Coliform - Antibiotic Resistance Clustergram
DISTANCE
Shaded cells = Broad Creek Okatee River Sites
* = Isolate that does NOT resemble STP
= Isolates with exact same MAR pattern
= Broad/Okatee SW samples
= Bluffton County SW samples
= STP
OK: T2; T3; I4 (3/3 = 100%)
BLF3 (1/2 = 50%)
% = number of sites with similar MAR patterns to STPs
BC: R4; R5; T2; T4; T6; I1 (6/7=86%)
BCR4; BLF2 ≠ STP

Broad Creek-Okatee River & LUCES Studies: Additional MAR Panel

Antibiotic	Conc. (µg/ml)	% Resistance
Nitrofurantoin	32	10
Cepahalothin	8	100
Aztreonam	8	70
Cefpodoxime	2	60
Cefazolin	8	30
Cefoxitin	8	40

(No resistance found in Ampicillin/Sulbactam, Ceftazidime, Piperacillin, Trimethoprim/Sulfamet., Ciprofloxacin, Cefuroxime, Ceftriaxone, Cefotaxime, Gentamicin, Tobramycin, Amikacin, Levofloxacin, Meropenem, Imipenem/Cilistatin)

Confirmed earlier contemporary MAR Panel for BC and OR



Okatee MAR Results 2002

LUCES Study: MAR Temporal Comparisons

Antibiotic	STPs	BC 1997	Okatee 1997	Okatee 2002
Ampicillin	2.1%	0.3%	0.0%	0.8%
Chlor. Tetra.	0.7%	0.6%	0.0%	2.5%
Kanamycin	0.0%	0.1%	0.0%	2.5%
Nalad. Acid	0.1%	0.1%	0.0%	3.3%
Neomycin	0.0%	0.0%	0.0%	0.0%
Oxy. Tetra.	1.1%	1.0%	0.0%	5.0%
Penicillin	5.4%	0.4%	0.9%	3.3%
Streptomycin	0.9%	0.0%	0.0%	3.3%
Sulfathiazole	0.7%	0.0%	0.1%	0.0%
Tetracycline	1.4%	0.8%	0.0%	5.0%
% MAR	12.3%	3.4%	1.0%	2.6%
# Antibiotics	8	7	2	8

*Percent Isolate Resisiance/Antibiotic

MAR: Regional Comparisons

Watershed	SITE MAR[1]		% Difference (DEV v. UNDEV)	Reference
	Developed	Undeveloped		
Florida (Appalachicola Bay)	25 (3.5)	13 (1.9)	47	Parreen et al., 1997
Maryland (Anacostia R., Annapolis Harbor Baltimore Harbor vs. Chester R., Miles R., Wye R., and Love Point)	9 (4.5)	2.8 (1.4)	69	Kaspar et al., 1990
South Carolina (Broad Creek vs. Okatee R.)	3	1	67	Van Dolah et al., 2000

[[1]( ) = Tidal Adjusted MAR)]

Broad Creek - Okatee River & LUCES Studies: Conclusions

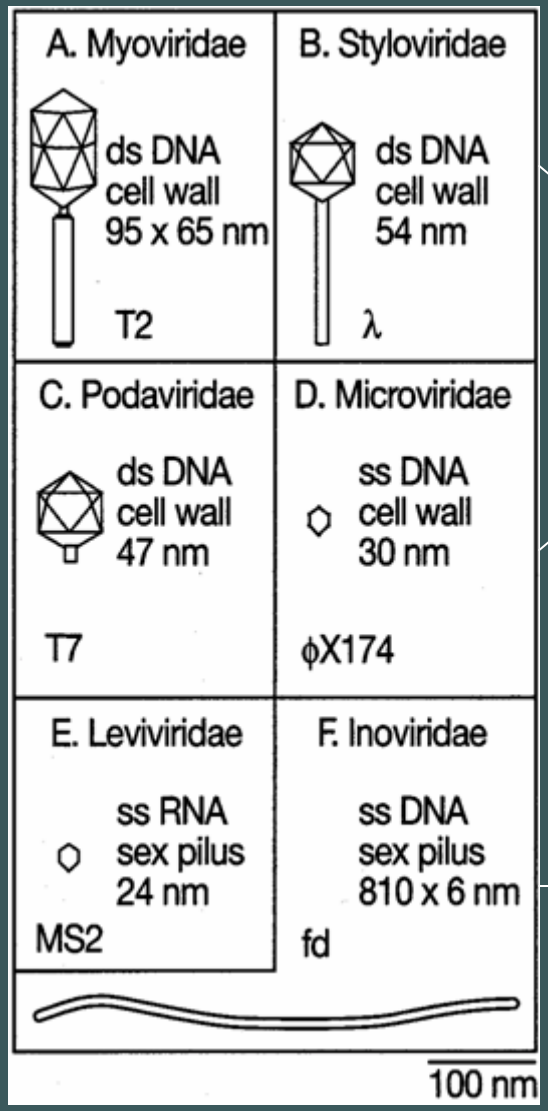
- High FC MPNs measured in both BC and OR.
- BC: 7 sites with high MAR which matched MAR patterns of WWTP (6/7 sites=85%).
- OR: 3 sites with high MAR which matched MAR patterns of WWTP (3/3=100%).
- GIS Analysis – High MAR regions in BC and OR were correlated with known pollution sources (WWTP, septic tanks, spray irrigation fields).


Saluda
Catawba
N
Pee Dee
Savanah
SC Impaired Watershed Study
Waccamaw

SC Impaired Watershed Study: Methods

- **Objectives**
  - **Determine source of FC causing impairment of water quality**
- **Methods**
  - **FC (API) → *E. coli* → MAR → Ribotyping**
  - **Coliphage (Somatic & Male)**
  - **F^+RNA Typing: Group 1 (animals & human)**
    Groups 2 & 3 (human)
    Group 4 (animal)

Coliphage Families



Somatic (F-)

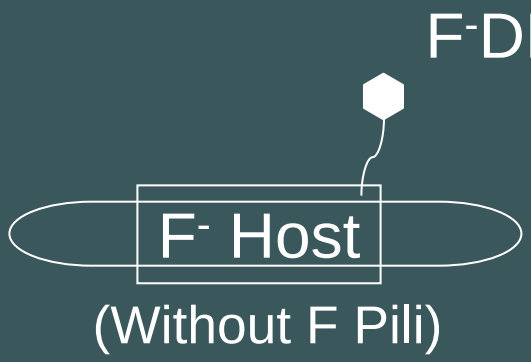
Infect through receptors on host cell wall

Male-Specific (F+)

Infect through receptors on host F pili

IAWPRC (1991). *Water Res*. **25**(5):529-545.

Types of Coliphages: Somatic (F^-)



← **Somatic**

Infect host through receptors on cell wall

Four Families

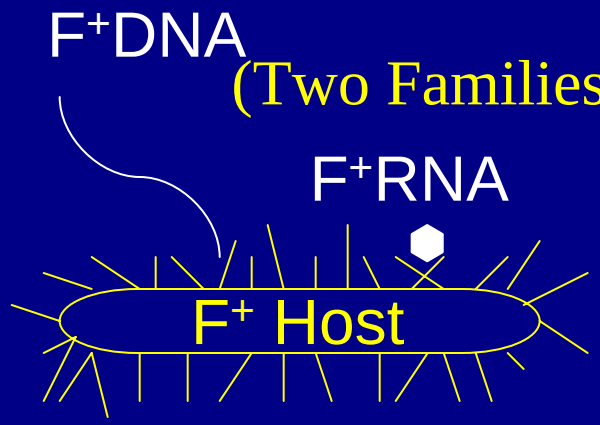
Siphoviridae *Myoviridae* *Microviridae* *Podoviridae*

Bar = 100 nm; First three photos by Fred Williams, EPA

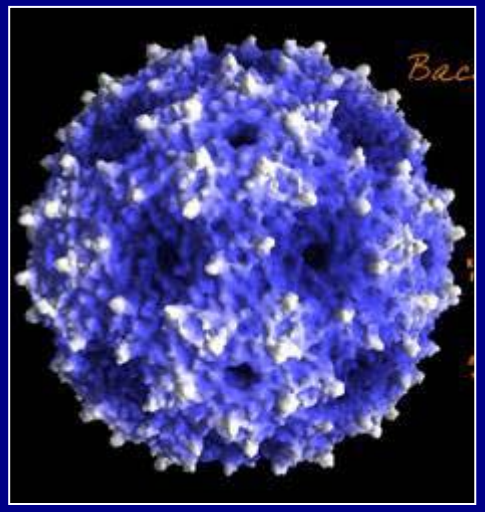
Types of Coliphages: Male-Specific (F^+)

Male-Specific →

Infect host through receptors on F pili



(With F Pili)



F^+RNA = *Levivirdae*

Bacteriophage MS2. Valegard et al. (1990). Licensed for use, Inst. for Molecular Virology.
(linked to http://www.bocklabs.wisc.edu/images/ms2.jpg). 6 May 2002.

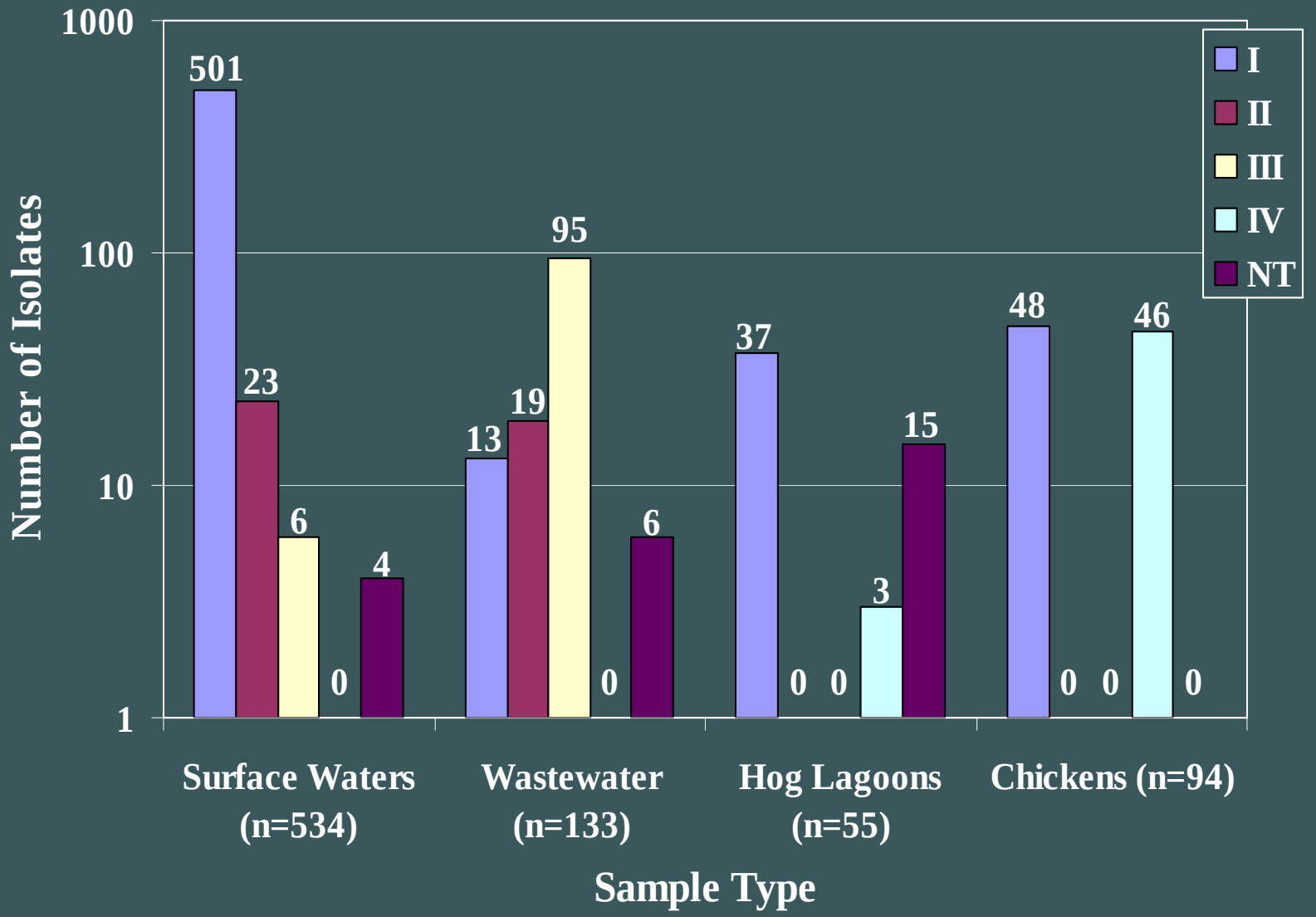
SC Impaired Watershed Study: MAR Results

Source	MAR Index	Antibiotics
WWTPs	8	COT
Chicken Farms	16	COT
Hog Lagoons	12	COT
Surface Water (All)	2	
- Savannah	2.9	PCKNOSSfT
- Catawba	0.9	APOT
- Saluda	2.8	APT
- Pee Dee	2.0	AP
- MD	3.9	APOT; COT
Other Animals[1]	0	-

1= (cows, dog, horses and birds)

Antibiotics Tested: Ampicillin (A), Chlortetracycline (C), Kanamycin (K), Nalidixic Acid (N), Neomycin (Ne), Oxytetracycline (O), Penicillin (P), Streptomycin (S), Sulfathiazole (Sf), Tetracycline (T)

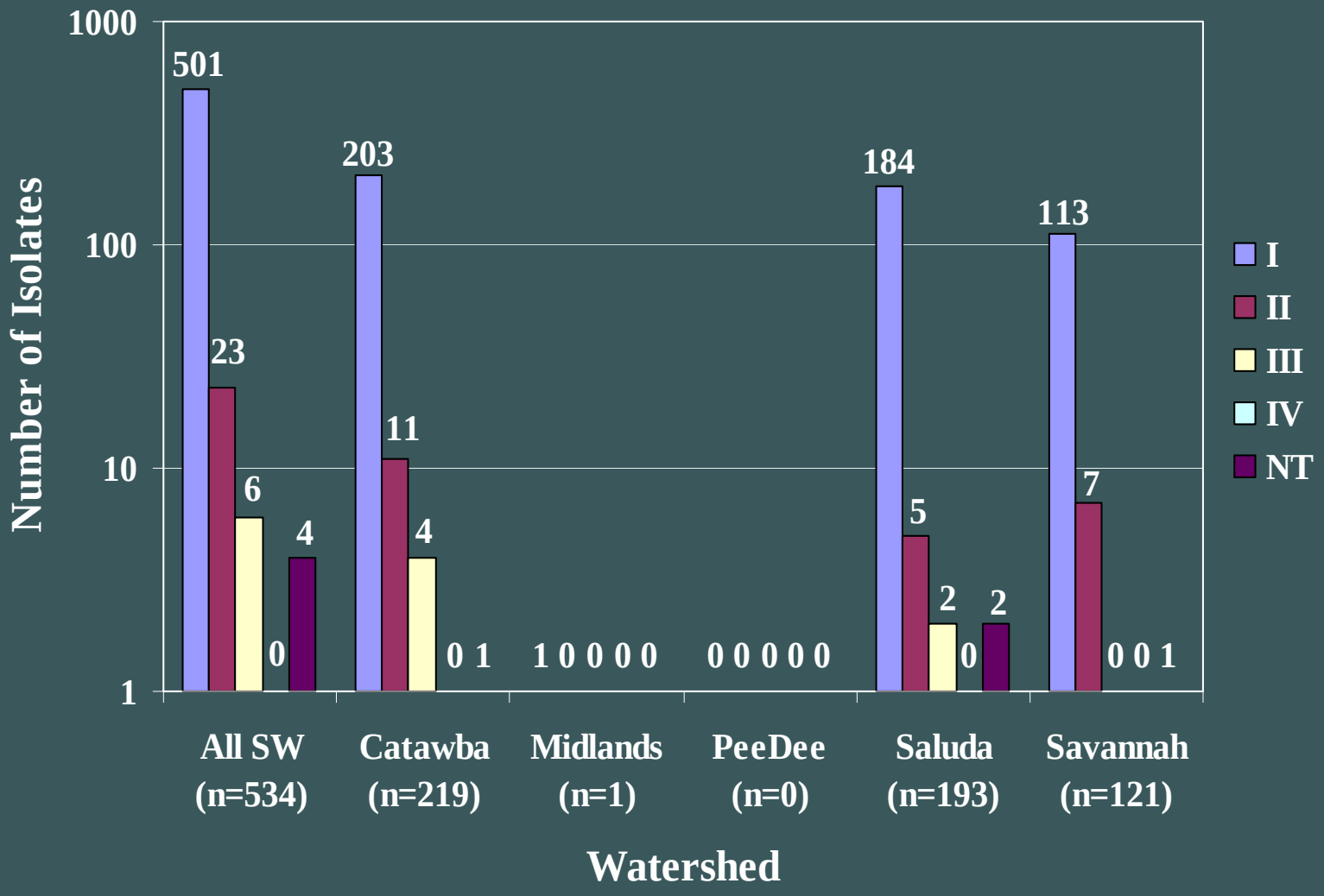
F+RNA Coliphage Typing Results



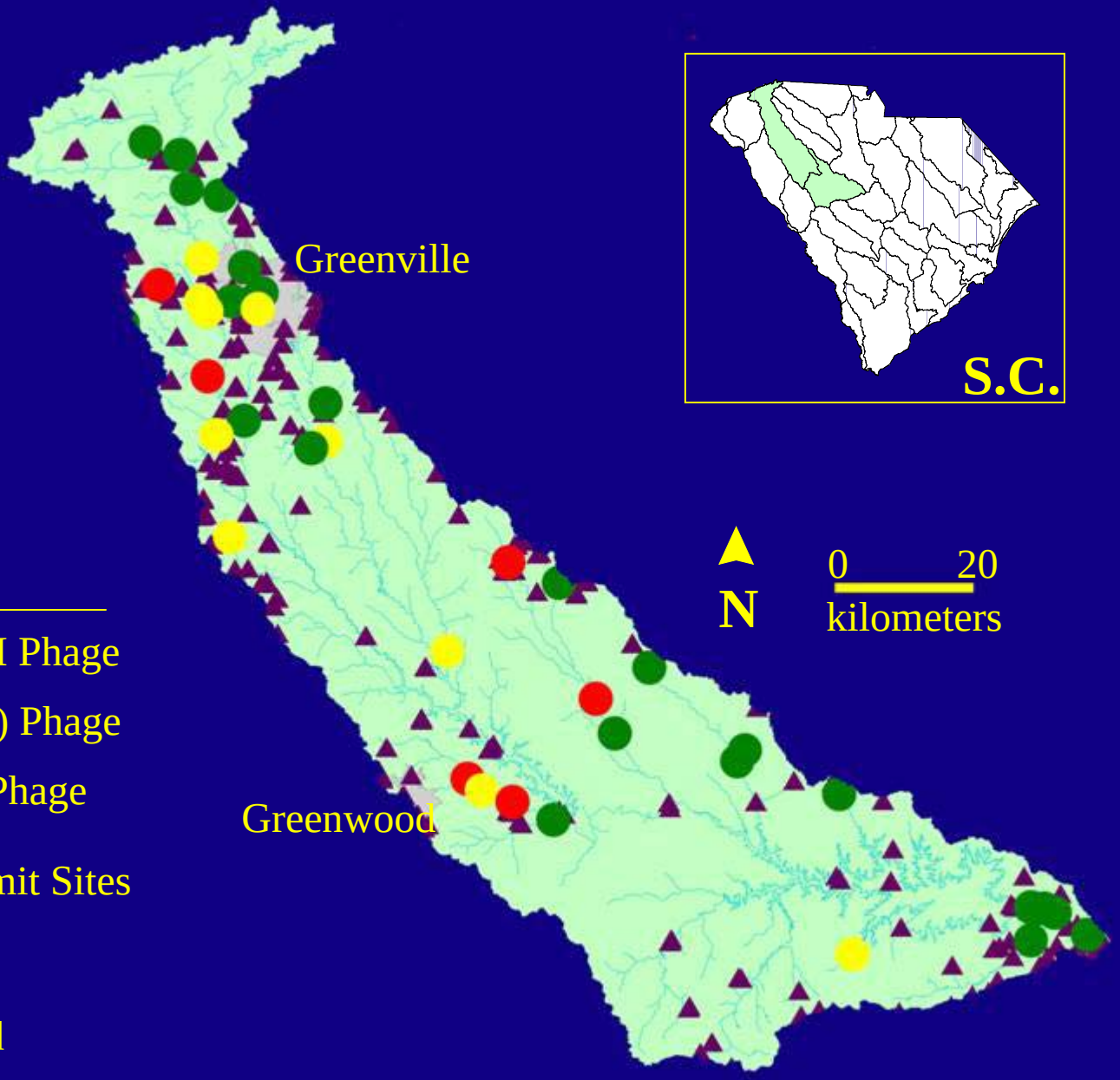
What is the origin of type I F^{+}RNA coliphages detected in municipal wastewaters?

http://www.softpawsk9.org/html/files.htm

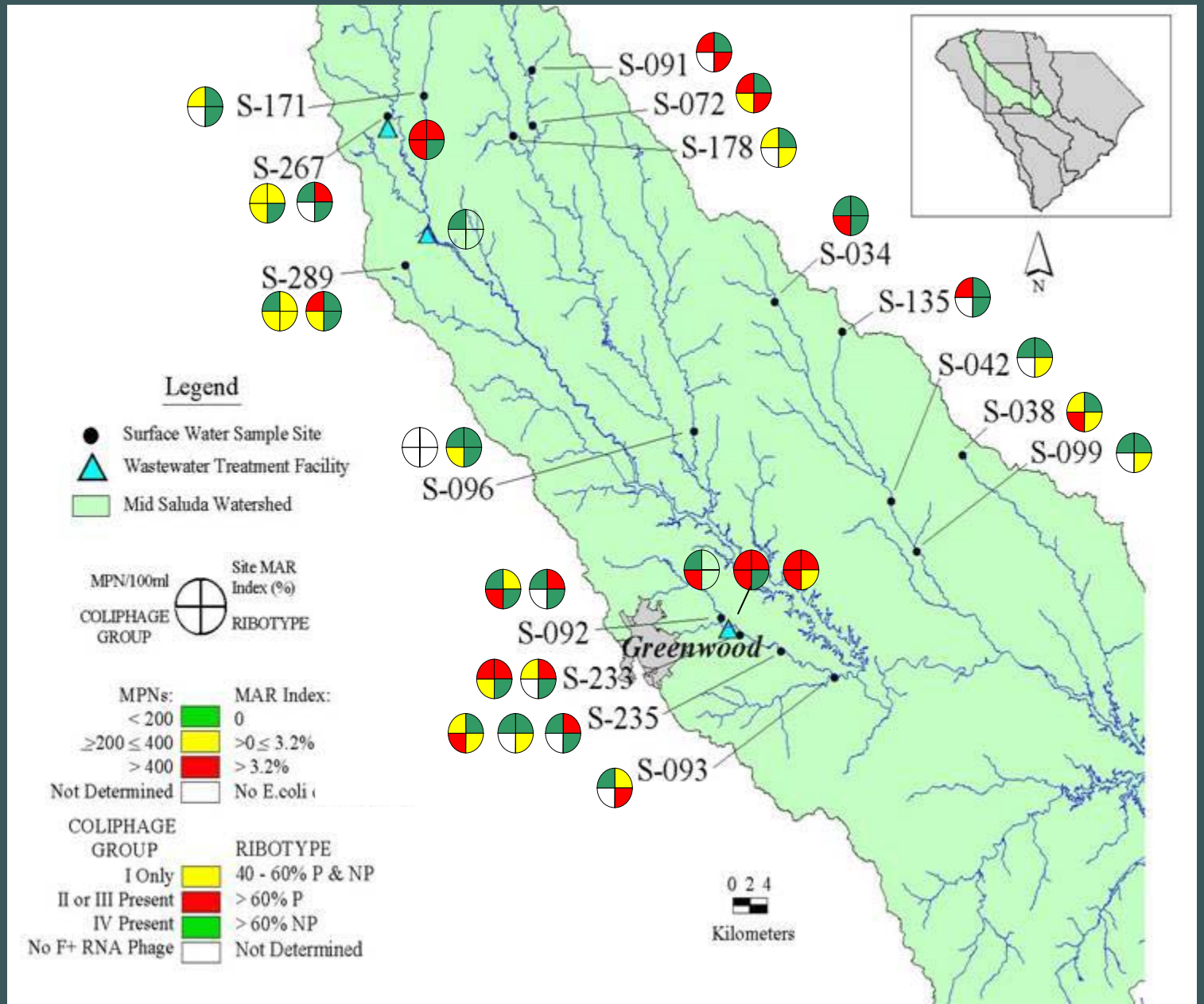
F+RNA Typing for Surface Waters.



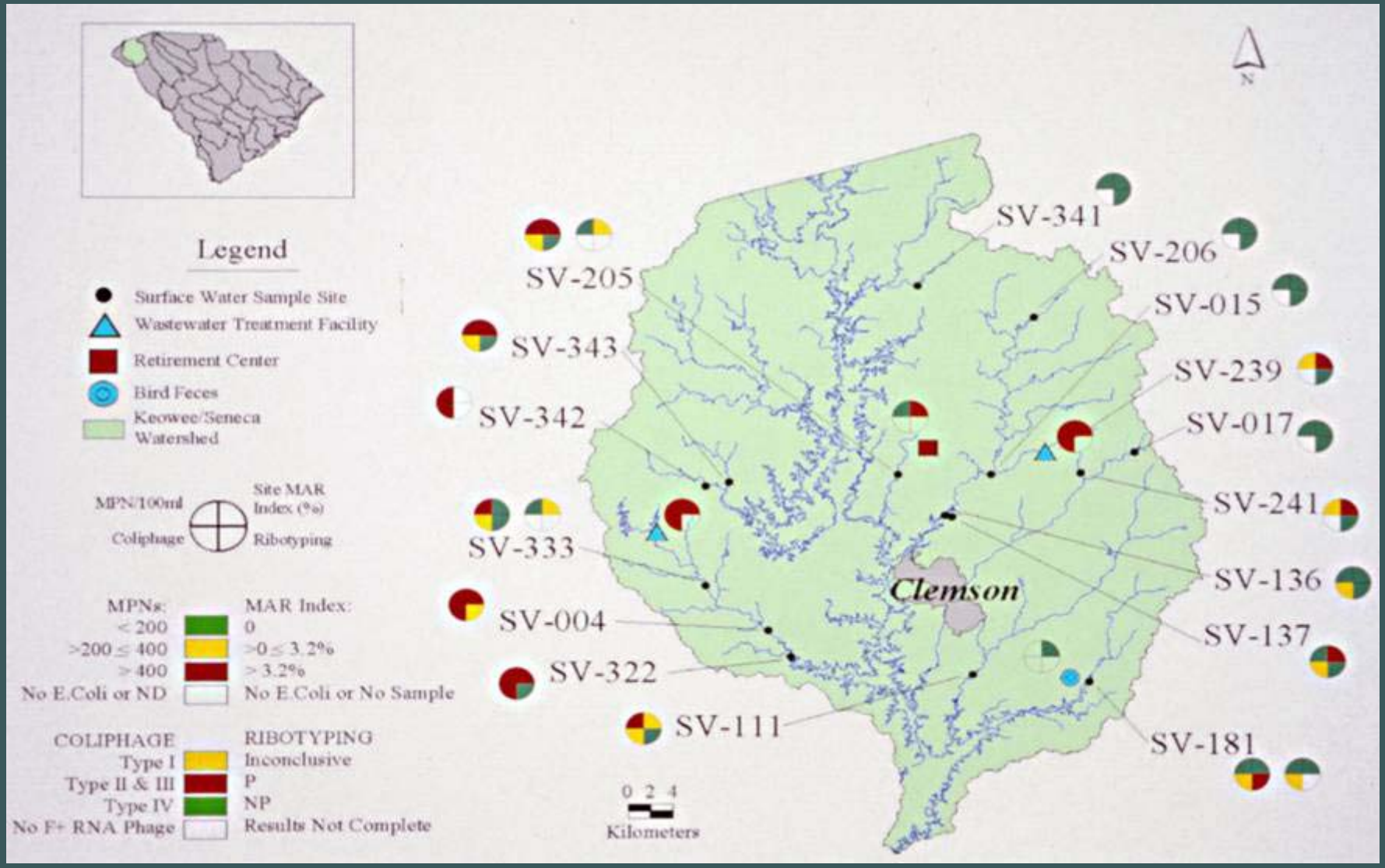
Saluda Watershed



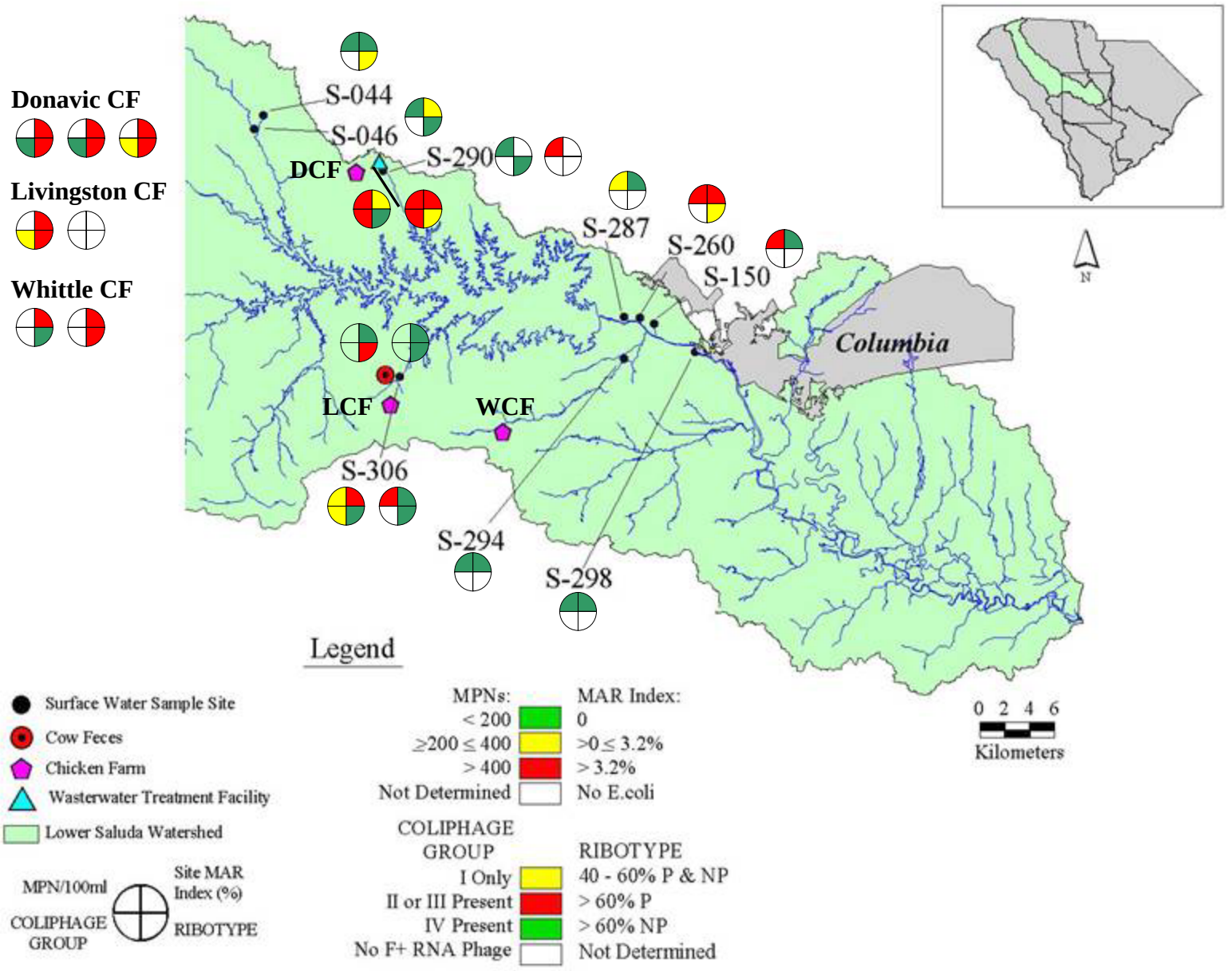
Middle Saluda Stations



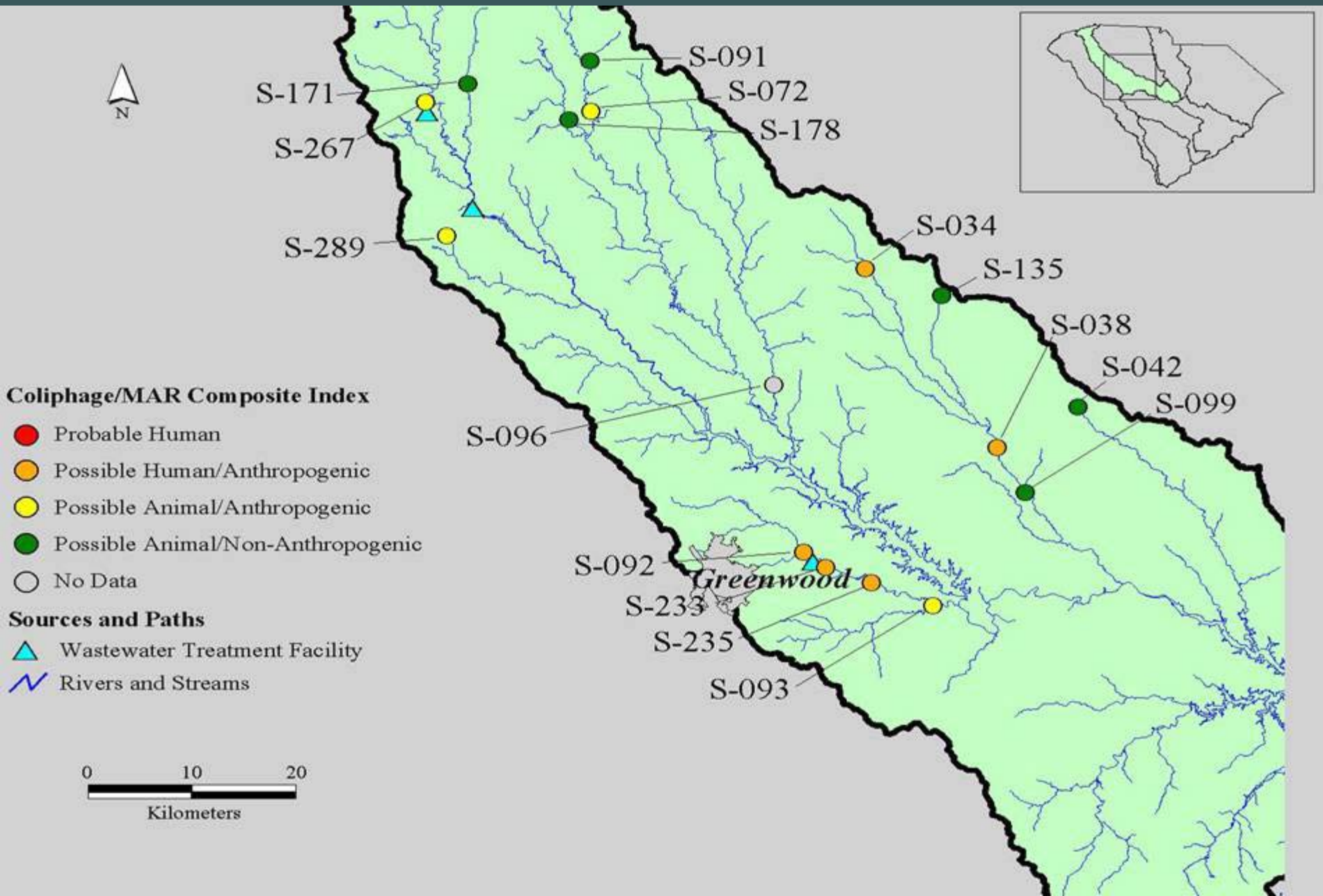
Coliform/Coliphage Assessment for the Keowee Stations



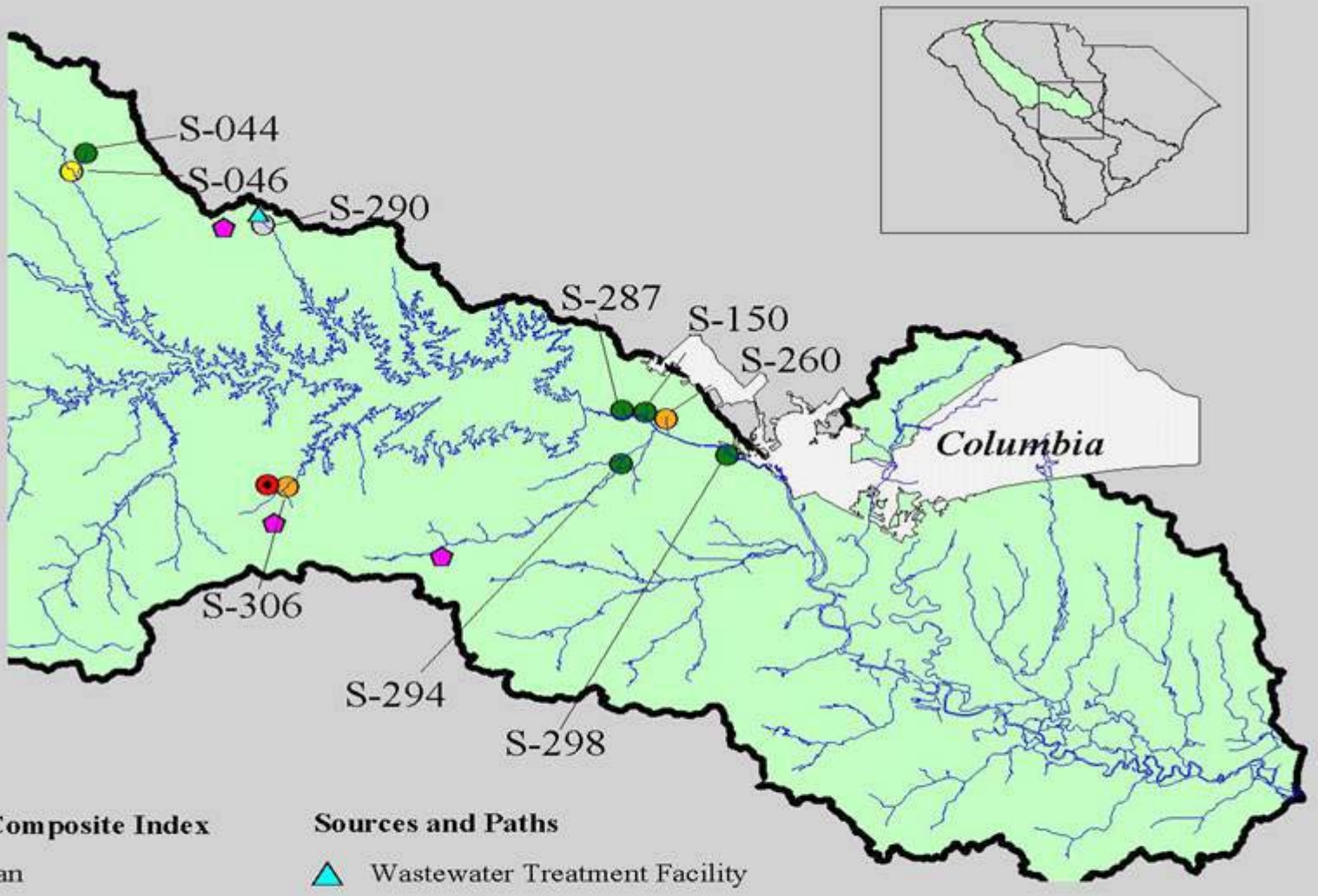
Lower Saluda Stations



Middle Saluda Watershed



Lower Saluda Watershed



SC Impaired Watershed Study: Conclusions

- High FC MPN's and coliphage levels appeared to co-occur in most watersheds and in known human pollution sources.
- High MAR and Groups 2 & 3 F^{+}RNA coliphages found at WWTP and sites downstream.
- Groups 2 & 3 F^{+}RNA coliphages not found at Chicken Farms and in other animals.

SC Impaired Watershed Study: Conclusions

- High FC and MAR found at sites adjoining WWTPs, Chicken Farms and Hog Lagoons.
- MAR (% Resistant to 1 Antibiotic): Chicken Farms (60%) > WWTP (28%) > SW (8%)
- MAR Index: Chicken Farms (16%) > Hog Lagoons (12%) > WWTP (8%) > SW (2%) > Animals (0%)
- Ribotyping analysis is incomplete.

CONCLUSIONS

- ***E. coli*** **was the dominant fecal coliform bacteria.**
- **High MARs were found in WWTP and Domestic Animal Sources with C-O-T and A-P-C-O-T Patterns.**
- **MARs varied among WWTPs.**
- **MAR was found more prevalently in surface waters adjoining know human pollution sources (WWTPs, septic tanks & land applied sewerage) than in pristine or NPS areas.**

CONCLUSIONS

- Coliphage Typing was useful, particularly when used in conjunction with MAR.
- Modeling of Land Use and Identification of Known Pollution Sources provided useful "Presumptive Models" of Bacterial loadings.
- Presumptive TMDL Models are useful to direct environmental management of bacterial pollution sources within a watershed.
- Future Studies: Link Multiple Methods

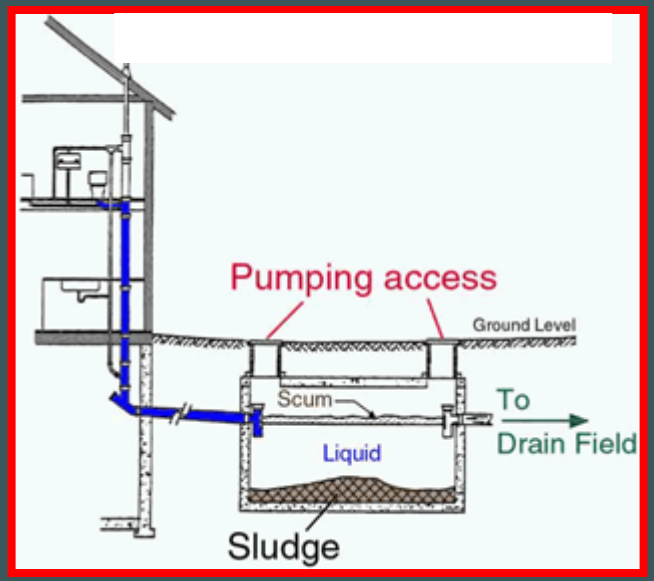
CONCLUSIONS

- PFGE and Ribotyping results were not conclusive and require local/regional specific database.
- Coliphage Typing was useful, particularly when used in conjunction with MAR.
- Modeling of Land Use and Identification of Known Pollution Sources provided useful “Presumptive Models” of Bacterial loadings.
- Presumptive TMDL Models are useful to direct environmental management of bacterial pollution sources within a watershed.
- Future Studies: Link Multiple Methods

Human Sources of Fecal Contamination

Municipal Wastewaters

Septic Tanks



Marinas

Animal Sources of Fecal Contamination

Livestock

Wild Animals

Pets